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MALAYAN AND SUMATRAN DISCOMYCETES

By EDITH K. CASH

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Collections made by E. J. H. Corner, mostly in 1929–33, have been worked out from manuscript notes and dried specimens by Edith K. Cash. Nine species of Operculatae and sixteen of Inoperculatae are recorded, including the following new species: *Dasyscypha brunneomaculata* Cash et Corner, *Encoelia singaporensis* Cash et Corner, *Helotium hinnuleum* Cash et Corner, *Mollisia isabellina* Cash et Corner, *Orbilia fici* Cash et Corner, *Sarcosoma umbrina* Cash et Corner, *Scleroderris sumatrana* Cash et Corner, *Spathularia heterospora* Cash et Corner and *Sphaerosoma malayense* Cash et Corner.

Types are in the Mycological Herbarium, U.S. Department of Agriculture, Beltsville. Duplicates are at the Cambridge University Herbarium, and, where material is sufficient, they will also be sent to the herbaria at Kew, Paris and Singapore.

Discomycetes were found disappointingly scarce in the Malayan lowland forests; little exploration was made in the mountains, but it seems that there is a richer flora in the pockets of wet forest which occur on and around the limestone hills in the north. This paper merely records such as were found by E. J. H. Corner, and is not a summary of all records.

OPERCULATES

1. *Cookeina sulcipes* (Berk.) Kuntze

Malaya: Slim River, 11 May, 1930, Corner *1261*; Pahang, Tembeling, 6 Nov., 1930, Corner *1266*.

2. *Galactinia retiderma* (Cooke) Le Gal

On burnt ground in secondary jungle, Malaya: Pahang, Tembeling, 17 Nov., 1930, coll. Corner, Singapore Field No. *24185*.

3. *Phaedropezia epispartia* (Berk. & Br.) Le Gal

On dead wood, Malaya: Pahang, Tembeling, 15 Nov., 1930, Corner *1269*, same locality, 8, 10 Nov., 1930, coll. Corner, Singapore Field Nos. *24166* and *24170*.

4. *Phillipsia carneicolor* Le Gal

On dead wood, Malaya: Pahang, Tembeling, 5 Nov., 1930, Corner *1253*; on sticks, same locality, 17 Nov., 1930, Corner *1272*.

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5. *Pulvinula etiolata* (Cooke) Le Gal

On ground in forest, Malaya: Pahang, Tembeling, 6 Nov., 1930, coll. Corner, Singapore Field No. *24159*.

6. *Pulvinula globifera* (Berk. & Curt.) Le Gal

Malaya: Singapore, Botanic Garden, April 1929, Corner *1240*; and Bukit Timah *1241*.

7. *Pyronema omphalodes* (Bull. ex Fr.) Fckl.

On newly burnt ground in forest, Malaya: Pahang, Tembeling, 18 Nov., 1930, coll. Corner, Singapore Field No. *24189*. This species was reported from Java by Boedijn (1940, pp. 272–5).

8. **Sarcosoma umbrina** sp.nov.

Apothecia substipitata, lente gelatinosa, sicca coriacea rugosaque, late cupulata usque irregulariter contorta, fusco-atra, margine glabro vel subdentata, extus rugosa, hyphis brunneis ad basim abundantibus praedita, hymenio umbrino; asci cylindrici, longestipitati, ad apices obtusi, octospori, 150–165 (–300) × 18–20μ; ascosporae uniseriatae, hyalinae, ellipsoideae rectae vel reniformiter subcurvatae, inaequilaterales, utrinque subangustatae, 1–2-guttulatae, 24–27 × 12–14μ; paraphyses numerosae, filiformes, ad apices inflatae et brunneae, in mazaedium brunneum coalescentes; hypothecium prosenchymaticum, fusco-brunneum; stratum intermedium 300–400μ crassum, hyalinum, aquoso-gelatinosum; cortex pseudoparenchymaticus, cellulis parvis dense compactis compositum, hyphis brunneis sparsis 5μ in diam. tectum.

Apothecia substipitate, tough gelatinous wrinkled and coriaceous when dry, widely cup-shaped to irregularly contorted, fuscous-black*, margin even or slightly dentate, exterior wrinkled, rough, with sparse black hairs more abundant at the base, hymenium Saccardo's umber to sepia; asci cylindrical, obtuse and blueing with iodine at the tips and gradually narrowed to a long stipe, 8-spored, 150–165 (–300) × 18–20μ; *ascospores* uniseriate, hyaline, ellipsoid, straight or slightly reniform, inaequilateral, slightly narrowed at the ends, smooth, with 1–2 large guttules and several smaller ones, 24–27 × 12–14μ; *paraphyses* numerous, filiform, brown and swollen at the tips, coalescing to form a brown mazaedium; *hypothecium* prosenchymatous, made up of dark brown parallel hyphae; *intermediate layer* 300–400μ thick hyaline, watery-gelatinous; cortex pseudoparenchymatic, composed of small dark brown, densely compact cells, roughened by loose clumps of cells and sparse brown hyphae 5μ diam.

On dead sticks in forest, Malaya: Pahang, Tembeling, 6 Dec., 1930, Corner *1270* (Type); same locality [no date] *1190*.

In the two species most closely resembling this fungus (*Sarcosoma rhytidii* (Berk.) Le Gal and *S. turbinatum* Wakefield) the spores are granulose, ornamented in the former by transverse bands and in the latter by granules arranged in longitudinal striations (Le Gal, 1953, pp. 204, 226). So far as can be determined, the spores in the Malayan specimens are completely smooth. They differ also from both of the species mentioned in the umber-coloured hymenium.

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* Colour readings are from Ridgway, R., *Color standards and color nomenclature*, Washington, D.C., 1912.

9. **Sphaerosoma malayense** sp.nov.

Apothecia 1–3·5 cm. lata, 0·5–1·5 cm alta, dispersa vel subgregaria, superficialia, gymnocarpica, primum patelliformia, deinde lobata et contorta, margine deflexo, a basi angusto affixa, hymenio vinaceo-brunneo usque umbrino, convoluto, extus pallidoria tenue villosa, demum cava, carne friabile, succoso; asci 300—360 × 21–27 μ, operculati, violenter dehiscentes, octospori, clavati, tenui-tunicati, operculo tenue sed conspicuo, 9–10 μ lato, recto vel recurvato; tunica in parte superiori iodicaerulescenti; ascosporae uniseriatae 12·5–13·5 μ in diam. (verrucis exclusis) globosae, hyalinae, dense verrucosae, verrucis parvis obtusis vel truncatis 1·2–2·5 μ altis, 2–3 μ latis ad basim, interdum in costis brevibus unitis, guttulam unicam grandem continentes; paraphyses numerosi, recti, subclavati, ad apices 6–8 (–10) μ infra 4–5 μ, apicibus guttulatis, hyalinis vel brunnescentibus, mucosis, simplicibus vel rare apicem versus furcatis, basim versus ramosae.

Apothecia 1–3·5 cm. wide, 0·5–1·5 cm. high, scattered or subgregarious, never caespitose, superficial, gymnocarpic, at first discoid, up to 6 mm. wide then lobed and contorted, the very obtuse and indistinct margin becoming deflexed, attached centrally by a narrow base: hymenium rich purplish or vinaceous brown, becoming purplish umber or umber with age, convolute: externally paler, or pale pink, becoming finely and thinly villous with secondary, white mycelium, whitish to the base, glabrous: flesh brittle, becoming hollow, juicy, pale pinkish or pale pinkish brown, whitish at the base. *Asci*, 300–360 × 21–27 μ, operculate, violently dehiscent, 8-spored, clavate, the spores uniseriate, thin-walled, the lid 9–10 μ wide, erect or recurved, thin but conspicuous: the wall of the distal half of the ascus turning pale blue with iodine, in the young asci most of the wall turning pale blue, the contents in the proximal two-thirds of immature asci turning reddish brown from glycogen. *Spores* 12·5–13·5 μ wide (spore body), globose, colourless, densely verrucose with small blunt or truncate warts 1·5–2·5 μ high and 2–3 μ wide at the base, the warts in some cases joined into short flanges 3–4 μ long, with one large, colourless gutta 8–10 μ wide. *Paraphyses* copious, straight, subclavate, 6–8 (–10) μ wide at the apex, 4–5 μ wide below, the tips immersed in pale pinkish brown mucilage, and the terminal cells with colourless or slightly brownish, faint guttae or sappy globules: simple above (or rarely forked), branched near the base, the proximal cells short, 20–50 μ long, the other cells gradually longer distally; the apical cell being the longest 120–220 μ, the intermediate cells 70–120 μ.

On bare earth, damp old and mossy bricks and mortar in shady places. Malaya: Botanic Gardens, Singapore, May 1929, Corner *1243*.

Structure. The flesh is composed almost entirely of a pseudoparenchymatous cortex, the medulla being unrecognizable because of the very limited marginal growth, and the subhymenium being very narrow, about 100 μ thick. The cortex is composed at first of interwoven and radiating, plectenchymatous hyphae, many of the cells of which swell up into subspherical or pyriform bladders 20–100 μ wide, though they are persistently narrowed at the septa (4–7 μ wide). These hyphae of inflated cells are directed more or less radially, being formed by the marginal growth of the apothecium, and between them are numerous uninflated, interweaving hyphae 4–8 μ wide with cells 25–55 μ long. All the hyphae are thin-walled and many of the cells, chiefly before inflation, contain opalescent glycogenous masses of suboleaginous globules. At the surface of the cortex there are many cylindric, blunt, thin-walled, excrescent, secondary mycelial hyphae 6–14 μ wide. The broad, blunt margin of the apothecium is composed of subcylindric hyphal ends 4–11 μ wide, the end cells of which are 35–80 μ long. The hyphae are closely packed, and slowly excrescent, forming a rather sudden transition to the mucilaginous paraphyses on the inside and a very gradual, broad transition to the cortex on the outside. The paraphyses arise from short subinflated cells 5–8 μ wide, in the subhymenium.

Development. Though primordia less than 700 μ wide were not found, the apothecium is clearly gymnocarpic. When the disk is 3–7 mm. wide, the asci begin to eject spores. The characteristics of the apothecium are the short period of upgrowth of the primordial shaft, causing the apothecium to be sessile; the slow, limited marginal growth and the great amount of intercalary growth in the hymenium causing the apothecium to be small with an arching, convoluted hymenium which disrupts the flesh despite the inflation of many of the cells of the hyphae. The hyphal ends (4–6 μ wide) of the primordial shaft become the central paraphyses. Ascogenous hyphae appear among the 'stalks' of these hyphae in primordia *c*. 1 mm. wide, and then develop centrifugally, the first asci to ripen being central. The ascogenous hyphae are 7–10 μ wide, with cylindric cells 25–50 μ long; only the terminal and subterminal cells from which the asci and new ascogenous hyphae arise respectively in the typical manner have nucleate contents. The spores are delimited as 9 μ spheres in biseriate order in the distal part of the ascus, over a length of 50 μ, when the ascus tips are 30–45 μ below the surface of the hymenium, that is, when they are 270–290 μ long. A tiny oil-drop appears in the spores when they are 10 μ wide, then generally a few more tiny oil-drops, one of which gradually enlarges into the gutta, the others probably coalescing with it. The spores become uniseriate as they develop in size and when full grown the warts are formed. The asci, before the spores are formed, have opalescent glycogenous contents in the basal two-thirds and, after the spores have formed, this portion gradually becomes vacuolate from above downward to the base. The marginal growth is typical but very slow compared with the intercalary growth of the hymenium which produces asci and paraphyses: probably marginal growth continues throughout the life of the apothecium.

Spore discharge is typical of Operculate Discomycetes. The asci are amyloid as in *Aleuria*, but more diffusely, and the spores are shot certainly to a height of 1·5 cm., as determined by experiment in May 1929.

Life of the apothecium must be 3–4 weeks or more. Six apothecia, 6–10 mm. wide, detached from the soil and kept under observation in the laboratory, spored continually for 11–14 days (at a mean temperature of 27° C.) These fruit bodies did not grow any larger so that the life of large apothecia must be considerably greater.

Sphaerosoma trispora McLennan & Cookson, which is similar in size and colour to the Malayan species, differs in the 3–4-spored asci and reticulate spores. The spores are alveolate in *S. alveolatum* McLennan & Cookson, as its specific name indicates, and are larger in those of *S. violaceum* Svrcek. The latter species differs also in the violaceous colour of the apothecia.

INOPERCULATES

1. *Catinella olivacea* (Batsch ex Fr.) Boud.

On wood, Malaya: Pahang, 9 June 1931, coll. Corner, Singapore Field No. *24864*. *Lagerheimia melanochlora* Boedijn appears to be synonymous with this species, judging from the description and illustration (Boedijn, 1940, pp. 366–7).

2. *Dasyscypha brasiliensis* (Mont.) Le Gal

On bark, Malaya: Pahang, Tembeling, 23 Nov. 1930, coll. Corner, Singapore Field No. *24195*.

3. **Dasyscypha brunneo-maculata** sp.nov.

Apothecia stipitata, dispersa, cupulata usque patellata, carnoso-membranacea, 0–2·5 mm. in diametro, margine involuto, stipite 0·5–1 mm. × 0·2 mm., roseobubalina usque fulvo-olivacea, pilis vinaceo-russis agglutinatis asperata, hymenio cinnamomeo; asci longe cylindrici, ad apices, angustati, brevi-pedicellati, octospori, poro iodi caerulescenti, 65–75 × 3·5–4 μ; ascosporae hyalinae, aciculares, unicellulares, guttulatae, utrinque acutae, 25–30 × 1–1·5 μ; paraphyses filiformes, eramosae, non inflatae, paulo ascos superantes; excipulum prosenchymaticum, subhyalinum, caespitibus pilorum rubro-brunneorum maculatum; pili septati, 90–105 μ longi, 3–4 μ in diam., infra subhyalini, ad apices usque 5–10 μ inflatae et dense incrustati, saepe in fasciculos agglutinati.

Apothecia stipitate, scattered, cup-shaped to patellate, margin inrolled, fleshy membranous, pinkish buff to tawny-olive, exterior roughened by clumps of vinaceous russet hairs, stipe concolorous, 0·5–1 × 0·2 mm., hymenium cinnamon; asci long-cylindrical, short stipitate, narrowed at apex, pore blue with iodine, 8-spored, 65–75 × 3·5–4 μ; *ascospores* acicular, one-celled, hyaline, guttulate, acute at both ends, 25–30 × 1–1·5 μ; *paraphyses* filiform, unbranched, slightly longer than the asci, not swollen at the tips; *exciple* prosenchymatic, of slender thin-walled subhyaline parallel hyphae; excipular hairs septate, subhyaline below, 90–105 μ long, subhyaline to yellowish and 3–4 μ below, heavily encrusted, dark and swollen to 5–10 μ at the tips, often agglutinated in clumps giving the exterior of the apothecia a spotted appearance.

On dead stems, Malaya: Fraser's Hill, Pahang, 14 May, 1930, Corner *1262*.

This fungus somewhat resembles *Dasyscypha appressa* (Cash) Le Gal from which it differs in much shorter asci and spores and in the swollen, brown-encrusted hairs.

4. *Dasyscypha congregata* P. Henn.

On petioles of *Arenga*, Sumatra: Brastagi, 4500 ft. elevation, 12 Sept. 1931, Corner *1258*.

5. *Dasyscypha inspersa* (Berk. & Curt.) Sacc.

On wood, Malaya: Pahang, Tembeling, 26 May 1931, Corner *1189*.

6. *Dasyscypha plebeia* Le Gal

On bark, Malaya, Pahang, Kota Glanggi, 5 Dec. 1930, Corner *1256*

7. **Encoelia singaporensis** sp.nov.

Apothecia singula vel caespitosa in stromatibus ex rimis corticis erumpentibus insita, –10 mm. latis, substipitatis, coriaceis, sicca friabilia, cupulata usque patellata, margine angusto lobato vel undulato, hymenio roseo-testaceo usque lateritio, extus concoloria, sicca subtiliter furfuracea, stipite 1–2 mm. longo, 0·5–1 mm. lato, obconico; asci anguste cylindraceo-clavati, octospori, ad apices rotundati, longe pedicellati, 55–75 × 5–5·5 μ; ascosporae uniseriatae, hyalinae, glabrae, oblongo-ellipsoideae, acutae subobtusaeve, 1–2-guttulatae, 5·5–7 × 3 μ; paraphyses hyalinae, subclavatae, supra 2–2·5 μ, infra 1–1·5 μ in diam., rare ad bases ramosae; cortex tenuis *c.* 50 μ crassus, cellulis pseudoparenchymaticis polygonis 5–15 μ latis pallide-rufescentibus compositus, hyphis 2–3 μ latis in superficiem usque 12 μ longis protrudentibus.

Apothecia erumpent between crevices of bark on a fallen branch, single or generally caespitose on small concolorous erumpent stromata, each producing two to many apothecia –10 mm. wide, substipitate, leathery, brittle when dry, disk exposed from the first, slightly concave, then plane to convex, lobed or undulate at the margin, pale light red to pinkish testaceous, later Pompeian red to brick red or ochre red, darker when dry, externally concolorous, smooth when moist, drying finely and minutely furfuraceous; margin narrow, scarcely incurved, whitish; stem 1–2 mm. long, 0·5–1 mm. wide, short, obconic, ill-defined; *asci* narrow cylindrical-clavate, 8-spored, not blueing with iodine, rounded at the tips, gradually narrowed to a long stipe, 55–75 × 4–5·5 μ; *ascospores* uniseriate, hyaline, smooth, oblong-ellipsoid, acute or subobtuse at the ends, 1–2-guttulate, 5·5–7 × 3 μ; *paraphyses* hyaline, subclavate, the apex straight, 2–2·5 μ wide above, 1–1·5 μ below, sparsely branched below; *cortex* thin, *c.* 50 μ wide, pseudo-parenchymatous, composed of polygonal cells 5–15 μ wide, with pale rufescent walls (giving the colour of the apothecium), a few hyphal ends of 2–3 μ wide up to 12 μ long projecting at the surface.

On wood, Malaya: Singapore, Mandai Road Forest, Oct. 1929, Corner *1244*.

8. *Helotium crocatum* (Mont.) Le Gal

On dead petioles, Malaya: Fraser's Hill, Pahang, 13 May, 1930, Corner *1268*.

9. **Helotium hinnuleum** sp.nov.

Apothecia stipitata, ad nervos hypophylla, cupulata usque applanata, carnoso membranacea, 0·5–0·7 mm. diam. pallido vinaceo-hinnulea, hymenio avellaneo vel cinnamoneo sicca rufo-brunnea, stipite supra pallido, infra atro, 0·2 mm. diam. et altitudine; asci cylindrico-clavati, apice applanati ad basim gradatim attenuati, octospori, 65–75 × 6–7 μ; ascosporae hyalinae, biseriatae, unicellulatae, longe ellipsoideae, utrinque subacutae, 11–13 × 2–2·5 μ; paraphyses filiformes, eramosae, flavidulae, ad apices 2 μ in diam.; excipulum pallide brunneolum, prosenchymaticum.

Apothecia hypophyllous on leaf veins, stipitate, cup-shaped to flat when fully expanded, fleshy membranous, 0·5–0·7 mm. diam., hymenium avellaneous to cinnamon, exterior smooth, margin, even, pale vinaceous fawn to vinaceous buff, drying vinaceous russet to cameo brown, stipe pale above, black below, 0·2 mm. diam. and height; *asci* cylindrical-clavate, flattened at the apex, pore blue with iodine, gradually narrowed at the base, 8-spored, 65–75 × 6–7 μ; *ascospores* hyaline, biseriate, one-celled long-ellipsoid, subacute at the ends, 11–13 × 2–2·5 μ; *paraphyses* filiform, unbranched, pale yellowish, 2 μ at the tips; exciple prosenchymatic pale brownish.

On leaf veins of unknown host, Malaya: Perlis Bukit Ketri, 24 Nov. 1929, Corner *1274*.

The fungus appears to be close to *Helotium epiphyllum* (Pers. ex. Fr.) Fr. as described by Le Gal (1953, pp. 342–4), differing in the black base of the stem and in the even smooth margin. The general dimensions of the Malayan fungus resemble those of *H. populinum* Fckl. found on leaves in Europe and North America, which, however, is different in colour. Of the various dark-stemmed species of *Helotium* and *Phialea* on leaves (*H. alboatrum* P. Henn., *H. lentum* Berk. & Br., *H. cecropiae* P. Henn., and *Phialea euspora* Rick) none agree with *H. hinnuleum* in colour or in the dimensions of the spores.

10. *Helotium velhaense* (P. Henn.) Le Gal

On twigs and dead wood, Malaya: Singapore, 1 Sept. 1940, Corner *1245*; Pahang, Fraser's Hill, 16 and 21 May, 1930, Corner *1260* and *1267*; Pahang, Tembeling, 9 Nov. 1930, coll. Corner, Singapore Field No. *24168*.

11. **Mollisia isabellina** sp.nov.

Apothecia sessilia, sparsa, tenui-carnosa, patellata, 0·5–1 mm. in diam., hymenio griseo-flavido, isabellino vel olivaceo, extus sordide citrino-avellaneo, margine nigro-brunneo, levi, involuto, angusto; asci cylindrici, tenues, apicibus subacutis, longe pedicellati, octospori; 65–70 × 3–4 μ; ascosporae biseriatae vel oblique uniseriatae, aciculares, hyalinae, utrinque acutae, 6–7 × 0·5 μ; paraphyses filiformes, apicibus non-inflatae; excipulum pallide brunneum, pseudoparenchymaticum cellulis tenui-tunicatis, hexagonis usque rotundatis, 6–12 μ latis, marginem versus elongatis, ad basim obscurioribus compositum; stratum interiore cellulis magnis, hyalinis, nitentibus compositum.

Apothecia sessile, scattered, thin-fleshy, shallow saucer-shaped to flat, hymenium light yellowish olive to deep olive buff, drying Isabella coloured to dark olive buff, 0·5 mm. diam., exterior citrine drab, margin dark brown, even, narrow, conspicuous; *asci* slender, subacute at the apex and long pedicellate, 8-spored, not blueing with iodine, 65–70 × 3–4 μ; *ascospores* biseriate or obliquely uniseriate, slender acicular, hyaline, acute at both ends, 6–7 × 0·5 μ; *paraphyses* hyaline, filiform, not swollen at the tips; *exciple* pale brown, pseudo-parenchymatic, composed of thin-walled, hexagonal to round cells 6–12 μ diam., darker toward the base of the apothecium and elongated toward the margin but not clavate; interior tissue made up of large, hyaline, shining cells.

On petioles, Malaya: Perlis, Bukit Lagi, Nov. 1929, Corner *1273*.

The asci are much longer than those of *Mollisia virídulo-mellea* Penz. & Sacc. from Java, a species rather similar to the Malayan specimen in the colour of the hymenium and the spore dimensions; *M. bromeliicola* P. Henn. from Brazil differs in colour.

12. *Ombrophila microspora* (Ell. & Ev.) Sacc. & Syd.

On cut surface of stump, Malaya: Pahang, Fraser's Hill, 20 May 1930, Corner *1265*.

13. **Orbilia fici** sp.nov.

Apothecia gregaria, sessilia, primum subglobosa dein cupulata usque patellata, gelatinosa, –3 mm. in diam., hymenio griseo-olivaceo, exteriore concolore, glabro, margine subtiliter dentato, sicco plicato, asci cylindrici, apice rotundati, brevi-stipitati, octospori, poro iodi caerulescenti, 35–40 × 2–3 μ; ascosporae filiformes, rectae vel curvatae, 10–11 × 0·5 μ; paraphyses hyalinae, filiformes, ascos superantes, apicibus sublanceolatis, 2–3 μ latae; excipulum hyalinum, pseudoparenchymaticum, cellulis tenuibus subglobosis usque angularibus ad basim 15–20 μ in diam., marginem versus minoribus et clavatis, composita.

Apothecia gregarious, sessile, subglobose, then deep cupulate to patellate, gelatinous, –3 mm. in diameter, hymenium drab to greyish olive, exterior concolorous, smooth; margin finely dentate, paler than the hymenium, becoming plicate on drying; *asci* cylindrical, rounded at the apex, short stipitate, 8-spored, pore blue with iodine, 35–40 × 2–3 μ; *ascospores* hyaline, filiform, straight or curved, 10–11 × 0·5 μ; *paraphyses* hyaline, filiform, outranking the asci, sublanceolate and 2–3 μ at the tips; *exciple* hyaline, pseudoparenchymatic, composed of thin-walled, subglobose to angular cells, 15–20 μ diam., at the base, smaller and clavate at the margin.

On bark of *Ficus irregularis*, Malaya: Singapore, Apr. 1935, Corner *1249*.

The long slender spores of *Orbilia fici* are close to those of *O. curvatispora* Boud. and *O. inflatula* Karst., but both of these species differ in the colour of the hymenium. *O. chlorina* Rick, which is close in the greenish yellow colour, is a polysporous fungus.

14. *Orbilia leucostigma* Fr.

Malaya: Singapore, Mandai Road Forest, July 1929, Corner *1247*.

15. **Scleroderris sumatrana** sp.nov.

Apothecia singula vel 2–3-caespitosa, sessilia vel substipitata, coriacea, cupulata, 4–9 mm. in diametro, 3–4 mm. alta, margine leniter plicato et crenato, extus rimosa, rugosa, furfuracea, fusco-atra, hymenio umbrino; asci cylindrici, longe stipitati, apice angustati, octospori, poro iodi caerulescenti, 150–175 × 7–8 μ; ascosporae aciculares, lunatae vel sigmoideae, irregulariter 2–3-seriatae, utrinque acutae, 50–55 × 1–1·5 μ; paraphyses numerosae, filiformes, simplices vel basim versus ramosae, granulosae, hyalinae usque pallide brunneae; excipulum crassum, atrum; textura strati mediani hyalina, prosenchymatica.

Apothecia single or 2–3-caespitose, sessile to substipitate, leathery, cupulate, 4–9 mm. diam., 3–4 mm. deep, margin somewhat plicate, slightly crenate, exterior fuscous-black, scurfy, rimose; hymenium Saccardo's umber to deep greyish olive; *asci* cylindrical, long-stipitate, narrowed at the apical pore, pore blue with iodine, 8-spored, 150–175 × 7–8 μ; *ascospores* acicular, crescent-shaped or sigmoid, irregularly 2–3-seriate, acute at both ends, 50–55 × 1–1·5 μ; *paraphyses* numerous, filiform, simple or branched towards the tips, granulose, waxy, hyaline to pale brownish;

exciple thick, cortex of heavy-walled black cells; medullary tissue hyaline, prosenchymatic.

On rotten wood in forest, Sumatra: Brastagi, 4500 ft. elevation, 11 Sept. 1931, Corner *1259*.

16. **Spathularia heterospora** sp.nov.

Ascomata clavata, stipitata, 3·5–6 cm. alta, ceraceo-gelatinosa, viscida, parte fertile 1–2 m. longa, olivaceo-brunnea, lobata usque gyrosa, cava, plerumque lateraliter compressa, spathulata vel cristaegalli similia, sinuata, lubrica, stipite 3–6 cm. longo, paulo compresso, costato sulcatoque, sursum incrassato, 4–5 × 8–16 mm. lato, glabro, olivaceo usque sepiaceo; asci cylindrici, longe stipitati, poro iodi caerulescenti, hymenium ad 40 μ supereminentes, usque octospori, 170–200 (–250) × 10 (13–16) μ; ascosporae multiseriatae, hyalinae, triformes, plerumque omnes in asco singulo praesentes: (1) 50–100 × 3–5 μ, cylindricae, subcurvatae vel flexuosae, multiguttulatae, apicibus obtusis; (2) breviores, 10–15 × 3–3·5 μ, saepe eguttulatae, plerumque rectae, apicibus subacutis; (3) rariores, longitudine intermediatae, apice uno hamatae et usque 7–10 μ incrassatae; paraphyses rectae, infra 1·2–2 μ in diam., apice filiformes vel breviter clavatae, usque ad 2–6 μ inflatae; cortex stipitis crassus, hyphis gelatinosis 1·5–3 μ in diam., medulla centrale hyphis flavo-brunneis cellulis 28–80 × 5–26 μ moniliformiter ad septa constrictis composita.

Ascocarp clavate, stipitate, 3·5–6 cm. high, waxy-gelatinous, the fruiting portion at the apex of the stem 1–2 cm. long, snuff brown to Verona brown lobed or gyrose, becoming hollow, usually flattened, spathulate or coxcomb-shaped, with more or less of a sinus on the flattened side, firm-waxy in texture, surface slippery; stem 3–6 cm. high, more or less compressed, ridged and furrowed, thickened upwards, 4–5 mm. wide in narrowest, 8–16 mm. in widest diameter, smooth, olivaceous darkening to warm sepia toward the base; *asci* cylindrical, projecting up to 40 μ above the hymenial surface, pore blue with iodine, 8-spored but one or more or sometimes all of the spores aborted, 170–200 (–250) × 10 (13–16) μ; *ascospores* multiseriate, hyaline, of three kinds: (1) 50–100 × 3–5 μ, cylindrical, slightly curved or flexuous, many guttulate, ends obtuse; (2) 10–15 × 3–3·5 μ, often lacking guttules, usually straight with subacute ends; (3) intermediate in size, more or less hooked and thickened up to 7–10 μ wide at one end, all three kinds of spores in a single ascus, usually four long and four short spores, the hooked spores rare; *paraphyses* straight, 1·6–2 μ wide below, filiform or short-clavate and 2–5 μ at the apex; *outer layer* of the stem thick, composed of hyphae 1·5–3 μ diam., with gelatinous walls; inner core of longitudinally arranged hyphae with firm, yellow-brown walls, divided into cells 28–80 × 5–25 μ, constricted at the septa and appearing moniliform; fungus with a strong odour of toffee or burnt sugar.

Malaya: Singapore Bukit Timah, May 1929 Corner *1242*; (*type*) Pahang, Tembeling, 16 Nov. 1930, *1271*.

The laterally compressed, tough gelatinous ascomata, with the hymenium decurrent on opposite sides of the stem, would place this fungus in *Spathularia*, in spite of the dark colour in which it differs from other species described. The three types of spores present in a single ascus is a character not previously noted in any species of *Spathularia*.

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THE BIOLOGY OF *HELICOBASIDIUM PURPUREUM* PAT.

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(With Plates 15 and 16 and 2 Text-figures)

Three isolates of *Helicobasidium purpurem* were shown, as originally isolated, to use both ammonium and nitrate N, as well as a mixture of amino acids. Two required thiamin and were partially deficient for some other growth factor or factors. The third behaved anomalously and its growth factor requirements were not determined. A cytological examination showed the vegetative mycelium of the isolates studied to be binucleate.

H. purpureum was shown to depend on a food base for growth through soil, which was little affected by a wide range of soil conditions. The fungus showed an affinity for continuous surfaces in soil. Pieces of carrot root stimulated growth once the fungus had made contact with them, but not otherwise.

Formation of mycelial strands from a food base appeared to constitute the normal growth habit of the mycelium in soil. A morphological study of strand development in natural (i.e. unsterilized) soil suggested that, under these conditions, there is some mutual attraction between the outgrowing hyphae of *H. purpureum*, so that hyphae coming together by chance tend to continue parallel growth in association. The coherence of strands formed in this way was increased by an interweaving growth of the main hyphae, by a binding action of short side branches and by anastomoses. The postulated attraction between hyphae presumed responsible for stranding did not, however, prevent branching of strands or their occasional disintegration into the constituent individual hyphae. Attempts to induce the formation of mycelial strands in pure culture were unsuccessful, so that it has not been possible to determine the nature of the stimulus responsible for stranding that operates upon hyphae growing through soil from a food base.

Survival of sclerotia of *H. purpureum* was poor in an acid soil, but was unaffected by a wide range of conditions in a slightly alkaline soil. Survival in the first mixed with sand was improved when this soil was rendered alkaline. Decreased longevity of sclerotia in the acid soil was associated with a high incidence of spontaneous germination.

INTRODUCTION

Violet root rot, caused by *Helicobasidium purpureum* Pat., is an easily recognized disease and has been known for over 200 years. The literature concerning it has been reviewed by Garrett (1946) and Whitney (1954). In Great Britain it is chiefly important as a disease of sugar beet (Hull & Wilson, 1946), although a severe outbreak was observed in 30 acres of carrots at Chippenham, Cambs., in 1955, approximately half the plants being affected. Considerable damage to carrots is reported in Canada (Whitney, 1954) and occasional instances of economic loss have been recorded in a variety of crops in many parts of the world.

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Garrett (1950) proposed a classification of the soil fungi on the basis of their ecological behaviour, giving the name 'root-inhabiting fungi' to those which are characterized by an expanding parasitic phase on the living host plant, and by a declining saprophytic phase after its death, the restriction of the saprophytic phase being caused by the competition of other soil micro-organisms. Observations have indicated that *H. purpureum* belongs to this group; this fungus is characterized by an ectotrophic growth habit, forms simple mycelial strands and survives in the absence of hosts as sclerotia.

Published reports of factors influencing severity of the disease, which have been summarized by Hull & Wilson (1946), present a confused picture and there is as yet very little information from experiments. This study was undertaken, therefore, to investigate the growth of the fungus in pure culture and also its growth and survival in the soil, in the hope that the information so obtained would lead to a better understanding of the disease.

ISOLATION AND CULTURE OF *HELICOBASIDUM PURPUREUM*

The isolates used in the present investigation were obtained from pieces of sugar beet and carrot root carrying infection cushions, the pieces being cut from areas showing no signs of rotting, surface-sterilized for 1 min. in 1:1000 mercuric chloride in 10% alcohol, washed in sterile water and plated-out on malt-extract agar. Growth was clearly visible after 6–8 days at 25° C., the colonies being 2·5 cm. or more in diameter after 3 weeks. In this way ten isolates were obtained from different sources and maintained on malt agar slopes under oil. Like those obtained by earlier workers (Buddin & Wakefield, 1927; Whitney, 1954) they showed considerable variation in colour, rate and habit of growth, production of sclerotia and tendency to sector.

The basidial stage was not observed in any of the isolates, although they were grown on a wide range of media under a wide range of conditions and, in spite of the fact that it is not known whether the fungus is homo- or heterothallic, were paired in all combinations on malt agar slopes. Production of conidia was desired, as this would have simplified inoculation of the liquid cultures used in nutritional experiments. Only at the end of the investigation, however, did the *Tuberculina* stage develop in a single subculture of one isolate. This stage was similar to that described by Buddin & Wakefield (1927), although the conidia measured 8–11 × 5–9μ, being smaller and more elliptical than those they described.

The three most stable isolates were selected for further study and designated A, B and C. A and B were isolated from sugar beet and C from carrot. The characteristic symptoms of the disease developed on Early Scarlet Horn carrots, inoculated with 8·5 mm. disks cut submarginally from 4 weeks malt-agar cultures (Garrett, 1946) of each of these isolates, after 6 weeks in a warm glasshouse.

CYTOLOGY OF THE VEGETATIVE MYCELIUM

Buddin & Wakefield (1927) studied the cytology of *H. purpureum*, and Ito (1949) studied that of *H. mompa* Tanaka. The vegetative mycelium of each was found to be usually binucleate and no clamp connexions were observed. The production of basidiospores was described but no descriptions of nuclei actually dividing, either mitotically or meiotically, have been given for either species

Isolates A, B and C were grown on plain, transparent 'Cellophane' (grade 300) on the surface of meat-malt agar (Buddin & Wakefield, 1924), pieces of mycelium from the edges of the colonies being floated off and fixed on to slides with egg albumin. Using Delamater's (1948) procedure 2 with basic fuchsin, the vegetative mycelium of each isolate was observed to be binucleate and to lack clamp connexions. The nuclei varied considerably in form.

The main difficulty in following the nuclear behaviour as successive cells were cut off from a hyphal tip was to recognize the dividing stages of the nuclei. Using this staining procedure no cases of the chromatin having resolved itself into individual chromosomes were demonstrated. Very large numbers of hyphal tips occurred in each preparation, but a first examination suggested that in none at all were the nuclei dividing, though, as they were taken from actively growing colonies, it seems probable that a proportion must have been in the process of division. It seems probable that the dividing nuclei looked similar to many of the resting ones in which the chromatin happened to be similarly disposed. They were only recognized to have been dividing, therefore, at the actual moment of separation or later.

From the stained preparations it appeared that each of the nuclei in a cell divided and that a septum formed between them at about the same time. Where anastomosis had occurred between two cells, a septum had appeared in one or the other or, rarely, in the bridge section, so that the resulting cells were binucleate with the nuclei apparently in their original pairs. However, it is not impossible that an exhaustive search or the use of other methods would reveal discrepancies.

NUTRITION IN PURE CULTURE

After trying a variety of media, Buddin & Wakefield (1924) found that *H. purpureum* grew best on a meat-malt agar and its growth on various media has been described by Whitney (1954). No satisfactory growth, however, has been reported on synthetic media and nothing has been recorded concerning its requirements for growth factors or its ability to use various sources of nitrogen and carbon.

Methods

In the following experiments the fungus was grown in liquid culture in 150 ml. conical flasks or, in some experiments, in 6 oz. medicine bottles, as described by Garrett (1953). All glassware was acid-washed and rinsed ten times with hot water and three times with distilled water. Ten ml. culture solution were allotted to each flask or bottle and, unless otherwise mentioned, these were then autoclaved for 15 min. at 15 lb. pressure. Each was seeded subsequently with a 4 mm. disk, cut with a No. 1 cork borer from the margin of a 4-week colony growing on water agar

(2 % Difco 'Bacto Agar' in glass-distilled water, 20 ml. per 9 cm. Petri dish). Each treatment was replicated three times and incubated at 25° C., the optimum temperature for growth as determined by previous workers (Buddin & Wakefield, 1924; Whitney, 1954). At the end of the culture period, the mycelium from each flask or bottle was filtered off on a previously dried and weighed 4·25 cm. Whatman No. 54 filter-paper, washed, dried overnight at 80° C., allowed to cool in a desiccator and weighed. All pH determinations were made with a Marconi pH meter.

Growth of the fungus in liquid culture consisted of a number of small, lumpy colonies, partly aerial and partly submerged. The dry weight of the mycelium was therefore, as Pelletier & Keitt (1954) point out in the similar case of *Venturia inaequalis*, an over-all result of the metabolic processes of a heterogenous mixture of physiological systems. However, since the various media used did not strikingly affect the morphology of the cultures, all the results are similarly conditioned by this complex type of growth.

Experimental work

Some preliminary experiments were carried out with isolate A to test the effect of the presence or absence of thiamin (0·1 mg./l.) and biotin (0·002 mg./l.), alone and in combination, and the ability of this isolate to use the nitrogen sources sodium nitrate, ammonium tartrate and Difco 'Casamino Acids', each added to a basal medium containing salts and 3 % glucose to give 0·02 % nitrogen.

The results were consistently poor and variable. Thiamin appeared to be essential for growth but the presence or absence of biotin was without effect. Good growth was obtained only with Difco 'Casamino Acids'* as the nitrogen source; growth on ammonium tartrate was consistently poor, and with sodium nitrate growth took place in some experiments and not in others. It seems probable that variants incapable of using this nitrogen source were present in the stock culture. No improvement was noted, either when the cultures were shaken at intervals or when 0·1 % of the non-ionic wetting agent 'Tween 80' was added. Results with isolate B were similar, although this isolate was consistently able to use sodium nitrate.

Experiment 1. As there was no information available concerning the pH range of the fungus in culture, an experiment was set up to investigate the effect of the initial pH of the culture liquid on the subsequent growth of the fungus.

Owing to the erratic behaviour of isolate A in the utilization of nitrate, isolate B was used in this experiment. The basal solution contained glucose (3 %), KH_2PO_4 (0·1 %), $MgSO_4.7H_2O$ (0·05 %), 0·25 ml./l. of a minor element solution,† similar to that used by Beadle & Tatum (1945), and 1 ml./l. of a growth factor mixture‡ (Pontecorvo, 1953). This solution was divided into three lots, to which were added 'Casamino Acids', ammonium sulphate + sodium fumarate (2 g./l.), and sodium nitrate, respectively, to give 0·02 % nitrogen. Each of these was divided into eight lots, which were adjusted to pH 2·0, 3·0, 4·0, 5·0, 5·5, 6·0, 6·5 and 7·0 respectively, with sodium hydroxide and hydrochloric acid. A preliminary trial had shown that, when the pH of the culture liquid was raised to 7, 8, or 9, it fell to below 7 on autoclaving and, therefore, no pH higher than 7 was tested. Each treatment was replicated six times, harvest of the first three replicates being made after 21 days, and of the second three after 35 days (Table 1).

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* A purified hydrolysed casein.

† $Na_2B_4O_7.H_2O$, 0·01 g.; $CuSO_4.5H_2O$, 0·1 g.; $FePO_4.2H_2O$, 0·2 g.; $MnSO_{4}5H_2O$, 0·2 g.; $Na_2MoO_4.2H_2O$, 0·02 g.; $ZnSO_4.7HO$, 2·0 g.; glass-distilled water to 250 ml.

‡ Folic acid, 0·02 g.; riboflavin, 0·2 g.; nicotinamide, 0·2 g.; *p*-amino-benzoic acid, 0·02 g.; pyricoxin HCl, 0·1 g; aneurin HCl, 0·1 g.; biotin, 0·0004 g.; Ca pantothenate, 0·4 g.; choline chloride, 0.4 g.; inositol, 0·8 g; glass-distilled water to 200 ml.

Table 1. *Effect of initial* pH *culture solution on growth of isolate B of* Helicobasidium purpureum

	pH of culture solution				Mycelial dry wt. (mg.)*	
	Initially	After autoclaving	After 21 days	After 35 days	After 21 days	After 35 days
Casamino Acids	2·0	2·0	2·0	2·0	1	1
	3·0	3·0	3·0	3·1	2	6
	4·0	3·9	4·0	4·3	9	37
	5·0	4·9	5·0	5·1	13	70
	5·5	5·5	4·8	5·2	35	110
	6·0	5·7	5·3	5·3	36	124
	6·5	6·1	6·0	5·7	45	120
	7·0	6·3	6·3	5·1	52	120
$(NH_4)_2SO_4$ + 0·2 % sodium fumarate	2·0	2·0	2·0	2·0	3	2
	3·0	3·0	3·1	3·4	9	19
	4·0	3·9	3·8	3·6	17	45
	5·0	4·9	4·1	4·1	38	89
	5·5	5·3	4·3	4·3	39	94
	6·0	5·6	4·5	4·6	37	102
	6·5	6·0	4·7	4·7	38	86
	7·0	6·3	6·1	5·9	7	9
$NaNO_3$	2·0	2·0	2·2	2·0	1	1
	3·0	3·0	3·1	3·0	1	3
	4·0	3·9	5·9	6·3	23	65
	5·0	4·9	6·2	6·3	35	65
	5·5	5·2	6·3	6·1	34	88
	6·0	5·7	6·4	6·1	37	72
	6·5	6·1	6·6	5·9	27	56
	7·0	6·2	6·9	6·4	14	27

* Mean of three replicates.

As a result of autoclaving, the pH of all solutions adjusted to pH 4·0, or above, fell and it can be seen that the higher the initial pH of the solution the greater was this fall in terms of pH units, ranging from 0·1 to 0·8. All solutions with an initial pH of 7·0 also showed a slight caramelization and precipitate. Hence the results obtained with these have not been taken into consideration in the interpretation of the experiment, as the differences observed between the amount of growth in these solutions and those of initial pH 6·5 are greater than could reasonably be expected to be caused by the small pH differences between such solutions after autoclaving.

Owing to the various complicating factors, it will suffice merely to note that the fungus made appreciable growth at pH 3·0 only with ammonium sulphate and that, in the series of initial pH 4·0, the best growth took place in the nitrate medium, presumably because of the subsequent rise in pH. From the results obtained with all three nitrogen sources, adjustment of the pH of the culture liquids to 5·5–6·0 before autoclaving is the most satisfactory general procedure.

Growth was considerably restricted below pH 4·0 in the ammonium medium and below pH 5·0 with 'Casamino Acids'. As growth on the nitrate medium raised the pH, no critical figure was obtained in this case, but it is obviously higher than pH 3·0. The better over-all growth in the ammonium medium compared to that in the preliminary experiments is ascribed to the improved buffering brought about by sodium fumarate.

Table 2. *Effect of nitrogen sources on growth of isolate B of* Helicobasidium purpureum

	% N in medium	Mycelial dry wt. (mg.)*		pH of culture solution	
		After 21 days	After 35 days	After 21 days	After 35 days
$(NH_4)_2SO_4$	0·01	—	30	—	2·4
	0·02	24	28	2·5	2·4
	0·04	—	29	—	2·4
$(NH_4)_2SO_4$ + 0·2 % sodium fumarate	0·01	—	78	—	5·1
	0·02	51	114	4·5	4·3
	0·04	—	91	—	4·1
Ammonium tartrate	0·01	—	54	—	2·9
	0·02	34	65	3·5	2·9
	0·04	—	28	—	4·1
Casamino acids	0·01	—	89	—	5·0
	0·02	68	128	5·3	4·7
	0·04	—	210	—	4·7
Alanine	0·01	—	57	—	4·8
	0·02	52	93	4·7	4·6
	0·04	—	127	—	4·5
$NaNO_3$	0·01	—	72	—	6·3
	0·02	17	57	6·3	6·1
	0·04	—	49	—	5·2
None	0·00	2	2	5·4	5·3

* Mean of three replicates.

Experiment 2. This experiment was designed to confirm the effect of buffering on the utilization of ammonium nitrogen and, at the same time, to compare growth on various nitrogen sources at three concentrations, 0·01, 0·02 and 0·04 % nitrogen. A complete basal medium with 3 % glucose was made up as in the previous experiment and all treatments at the 0·02 % nitrogen level were replicated six times, so that a harvest could be made after 21 as well as after 35 days. All culture solutions were adjusted to pH 6·0 before autoclaving and isolate B was again used. Results are given in Table 2.

Table 2 shows that, as the amount of growth decreased down the series ammonium sulphate + sodium fumarate, ammonium tartrate, and ammonium sulphate, so did the final pH of the culture solution. This indicates that growth was inhibited by a rapid fall in pH except where the solution was adequately buffered. It is also clear that the optimum level of nitrogen, under the conditions of the experiment, varied with the different sources, and, therefore, that results of carbon:nitrogen ratio studies could be expected to differ according to the source of nitrogen used. At the 0·02 % level of nitrogen, however, there was little difference between the amounts of growth with ammonium sulphate + sodium fumarate, 'Casamino acids', and alanine, though growth with sodium nitrate was poorer. Nevertheless, the 0·02 % nitrogen level seems the best choice for experiments on growth factor requirements.

Table 3. *Effect of nitrogen source and growth factors on growth of* Helicobasidium purpureum, *isolates A and B*

			Mycelial dry wt. (mg.)*		pH of culture solution	
			After 21 days	After 35 days	After 21 days	After 35 days
Isolate A	Casamino Acids	None	6	12	4·9	4·2
		Thiamin	22	69	5·4	5·1
		Mixture	33	128	5·8	5·0
	$(NH_4)_2SO_4$ + 0·2 % sodium fumarate	None	5	7	5·3	5·2
		Thiamin	16	91	4·7	3·9
		Mixture	30	114	4·5	4·0
	$NaNO_3$	None	3	8	5·4	4·7
		Thiamin	3	4	5·6	5·7
		Mixture	5	8	5·7	6·1
Isolate B	Casamino Acids	None	5	4	4·4	3·8
		Thiamin	45	127	5·7	5·3
		Mixture	67	144	5·8	5·0
	$(NH_4)_2SO_4$ + 0·2 % sodium fumarate	None	8	11	4·8	4·4
		Thiamin	26	109	4·8	4·4
		Mixture	83	159	3·9	4·7
	$NaNO_3$	None	5	12	5·2	3·7
		Thiamin	21	77	6·6	6·3
		Mixture	62	114	6·5	6·2

* Mean of three replicates.

Experiment 3. An experiment was now set up to test the ability of isolates A, B and C, to use three nitrogen sources in the presence or absence of growth factors. These were used at the same concentrations as before; on the evidence of the preliminary experiments, biotin was not tested either separately or with thiamin, but only as a constituent of the growth factor mixture. Results for isolates A and B are shown in Table 3. Isolate C did not make appreciable growth in any of the media.

Isolate A was again unable to use nitrate. Isolates A and B both required thiamin and both made more rapid growth in the presence of the growth factor mixture, indicating that they are partially deficient for some other factor or factors. On the basis of the final pH figures it is considered that the pH had little or no effect in producing differences, but it is interesting to note that media containing no growth factors were much reduced in pH, in spite of the fact that virtually no growth took place.

In another experiment in which inoculum disks were cut submarginally from 4-week meat-malt agar colonies, isolate C grew almost as well as isolate B with all three nitrogen sources in the presence of the growth factor mixture, though growth was again negligible where water agar inoculum was used. Isolate A again made no growth in the nitrate medium and it is clear that this isolate had lost its ability to use this source of nitrogen. The behaviour of isolate C, however, is not fully understood. It is not likely that its failure to make appreciable growth from water agar inoculum was caused by the toxicity of the media to the attenuated mycelium, since visible growth of normal hyphae appeared. It seems probable either that this isolate required growth factors present in meat-malt agar and not present, or present in insufficient quantities, in the growth factor mixture, or that growth on water agar had selected out a strain unable to make satisfactory growth in the culture solutions. Conflicting results were obtained from experiments designed to elucidate this problem and, owing to continued difficulties, this work was discontinued. Greater success might perhaps have been obtained in investigating the growth factor requirements of isolate C had the serial subculture method of Jennison, Newcomb & Henderson (1955) been used.

After completion of the foregoing experiments the pathogenicity of isolates A, B and C, as subcultures from the final nutritional experiment, was tested, using Early Scarlet Horn carrots growing in Cambridge Botanic Garden soil, as described in the section on isolation and culture of *H. purpureum*. After 4 weeks, extensive infection and superficial mycelial growth had developed from the inoculum disks of isolates B and C. Even after 8 weeks, however, no infection nor any superficial mycelium had developed from the disks of isolate A. Subsequent tests with similar inoculum disks placed on slides buried in the same soil, held at 60 % moisture-holding capacity, showed that this isolate did not grow out into the soil, whereas considerable mycelial growth was made by the other two isolates. It is clear, then, that isolate A, even if it had not lost its intrinsic pathogenicity, had lost its ability to cause infection because it was no longer able to grow through soil or over the surface of carrot roots. It is not known whether there was any connexion between its inability to grow through unsterile soil and loss of ability to use nitrate nitrogen.

The general conclusion that can be drawn from the nutritional experiments is that isolates B and C, and isolate A as originally isolated, could use both nitrate and ammonium nitrogen and a mixture of amino acids (Casamino Acids). Isolates A and B required thiamin and were also partially deficient for some other growth factor or factors. Owing to difficulties described above, the growth factor requirements of isolate C were not determined.

Development and behaviour of the mycelial strands

As pointed out by Garrett (1956*a*) 'the significance of the ectotrophic growth habit in root-inhabiting fungi lies in its manifestation of an ability for continuous and indefinite spread over the host root system', in spite of host resistance to infection. In the case of *H. purpureum*, which seems able to grow only slowly inside the host root and for a short distance from the point of penetration, such a growth habit is a very great advantage, enabling the fungus to colonise a root relatively quickly by growing over its surface and penetrating it, by means of single hyphae, at a large number of closely spaced points. Owing to host responses to infections, such as described by Whitney (1954), it is clear that a progressive infection could be established in no other way.

As infection must depend upon the ability of the fungus to grow through the soil, at least over the surface of the host, a study of the mycelial strands and of factors affecting growth in soil was undertaken.

Factors affecting growth and mycelial strand production in soil

Methods. In preliminary experiments using agar inocula of uniform size and age placed on glass slides buried in soil, as described by Garrett (1946), satisfactory growth and mycelial strand production occurred.* It was decided, therefore, to use this technique to study the effect of various factors on growth and strand production in soil.

For this purpose 8·5 cm. disks cut submarginally from 21–28-day cultures of isolate B on 6% malt agar (20 ml. per 9 cm. Petri dish) were used. Soils were prepared and their moisture-holding capacities determined as described by Garrett (1956*a*). It was found with slides buried in jars of soil that a considerable proportion of the mycelium was lost when they were removed. For this reason growth was observed in *situ* on the undersurfaces of slides pressed firmly against the surfaces of soils in Petri dishes. Unless otherwise mentioned, the inoculum disk was placed at one end of the slide, mycelial side against the glass, and pressed into the air-dry soil, which was then brought up to the desired moisture content. The dishes were incubated at 22·5° C. in batches of twelve, each batch contained in a polythene bag secured with an elastic band. Weighings showed that the amount of water lost during experiments was insufficient to justify subsequent additions. The method has the advantage that mycelial growth can be observed under the binocular dissecting microscope without disturbing the slide, and so measurements can be made as often as required on each slide.

The extent of mycelial growth along the slide from the centre of the inoculum disk was measured; at the same time, a count was made of the number of mycelial strands crossing a semicircle of radius 1 cm. from the centre of the inoculum disk, and the thickness of each strand was measured.

An attempt was made to replace the Petri dish technique by growing the fungus in soil tubes, as described by Garrett (1954) for *Psalliota hortensis*, but the tubes did not prove as satisfactory, owing to the shrinkage of the inoculum disk and the difficulty of observing the fine strands and hyphae, which are brown, on the curved soil-glass interface. Attempts to observe the growth arising from inoculum disks placed between sheets of Cellophane pressed on to or buried in soil, as used by Dobbs & Hinson

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* Dr Garrett has informed me that in the two experiments he made with slides, he obtained good mycelial growth of *H. purpureum* over the slides but no production of mycelial strands; the absence of strands in his trials has not been explained.

(1953), were discontinued, because the Cellophane became overrun with other organisms within a few days.

Effect of the food base. The dependence of *H. purpureum* on its food base for growth through soil has been suggested by observations and by an experiment performed by Whitney (1954), who, by using a series of agar inoculum blocks of decreasing size, showed that a certain inoculum potential was necessary to initiate infection on carrots; a few strands grew out from the smallest blocks but no infection developed. He concluded that, in the absence of an adequate food base, *H. purpureum* cannot initiate infection and that, until infection becomes well established, the supply of food appears to be a limiting factor for further growth.

The experiment reported here was modelled on one performed by Blair (1943) with *Pellicularia filamentosa*.* Cambridge Botanic Garden soil (a medium loam, pH 7·5), a mixture of one volume of the same soil with three of sand (pH 7·5), and sand (pH 6·5) were used (twelve dishes of each); the inoculum disks were removed from under the ends of six slides of each series after 17 days. The soils were brought to 60 % m.h.c. with distilled water. The results are shown in Table 4; those for sand are omitted, since growth progressed for no more than a few mm. in any dish and no strands formed.

Table 4. *Effect of inoculum removal on growth of* Helicobasidium purpureum *through soil*

	Growth after 17 days*			Growth after 27 days*			Growth after 35 days		
Soil and treatment	Extent (mm.)	No. of strands	Thickness of strands (μ)	Extent (mm.)	No. of strands	Thickness of strands (μ)	Extent (mm.)	No. of strands	Thic ness stra (μ
Whole soil	25	1·0	28	53	4·0	28	71	4·8	30
Whole soil, inoculum removed after 17 days	26	2·7	24	39	2·8	24	42	3·0	24
Soil:sand	25	1·5	32	53	3·5	30	66	3·0	28
Soil:sand, inoculum removed after 17 days	22	2·5	26	38	2·7	22	45	3·2	25

* Mean of six replicates.

It can be seen that, where the inoculum was not removed, the mycelial growth had increased in extent considerably at each successive reading and the number of strands had also increased. Where the inoculum was removed strand numbers remained much the same. This indicates that further growth after 17 days was at the expense of the food reserves of the mycelium which had already grown into the soil. Also there appears to be no difference between the growth in soil and in soil:sand. Nevertheless, soil obviously has some stimulating effect on *H. purpureum*, as illustrated by its failure to make satisfactory growth into sand, an effect similar to that observed by Garrett (1956*b*) for growth of rhizomorphs of *Armillaria mellea*. *H. purpureum* also failed to make satisfactory growth into crushed pumice

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* Imperfect stage = *Rhizoctonia solani*.

(pH 7·2). There was no improvement when unsterile sand and pumice were moistened with carrot root exudate, soil extract, a solution of salts and growth factors, or a solution of salts, growth factors and sodium nitrate.

Effect of host roots. Owing to the slow growth of the fungus and the fast growth of rootlets, it is difficult to devise a technique to demonstrate whether or not host roots attract and stimulate the fungus. Kerr (1956), by planting seedlings with their roots enclosed in Cellophane bags in soil inoculated with *Pellicularia filamentosa*, showed that this fungus rapidly aggregated on the Cellophane next to the roots of radish and lettuce. He concluded that the aggregation must have resulted from diffusion of a substance or substances through the Cellophane. Such experiments, however, took only 3 days, whereas, when similar ones were attempted with *H. purpureum* and carrot roots, the Cellophane had rotted long before any growth of the fungus was visible. Other methods, therefore, were tried in an attempt to throw some light on the effect of the host root on the fungus in its pre-penetration phase.

The effect of pieces of mature carrot root on growth and strand production in dishes of Botanic Garden soil:sand (1:3) at 60% m.h.c. was studied as follows. In one series of dishes the agar inoculum disk was placed under the centre of a piece of microscope slide 2 cm. long; a second series was prepared similarly except that a thick transverse slice of carrot root was placed in each dish touching the glass-slide; a third series was prepared similarly to the second, except that the inoculum disk was placed under the end of the slide so that it touched the carrot. Measurements were made as before, but, where the inoculum disks were under the centres of the slides, separate measurements were made of the growth towards both ends (Table 5).

Table 5. *Effect of pieces of carrot root on growth of* Helicobasidium purpureum *in soil: sand mixture*

		Growth after 17 days*			Growth after 27 days*		
	Direction of growth	Extent (mm.)	No. of strands	Thickness of strands (μ)	Extent (mm.)	No. of strands	Thickness of strands (μ)
oculum in centre of ide, no carrot	Left	17	0·5	22	>20	3·5	25
	Right	18	0·5	23	>20	2·9	27
oculum in centre of ide, carrot at end	Towards carrot	20	1·0	24	>20	8·4	31
	Away from carrot	19	1·1	22	>20	4·9	29
oculum at end of slide uching carrot	Away from carrot	21	0·7	25	>20	2·7	25

* Mean of twelve replicates.

After 17 days, growth was much the same in all dishes. Where the inoculum disk was next to the carrot there was already vigorous surface growth on the carrot, but growth from the inoculum away from the carrot was unaffected and, as far as could be seen, no hyphae were growing towards the carrot other than fortuitously. Where the inoculum disk was in the centre of the slide, hyphae were on the point of touching the carrot at this stage.

After 27 days, however, the picture was considerably different. The hyphae had grown beyond the ends of the slides in all cases, so no comparisons could be made as far as distance of growth was concerned. Where the inoculum disk was next to the carrot, growth away from the piece of root still seemed to have been unaffected, even though infection had taken place in ten of the twelve replicates. Where the inoculum disk was at the centre of the slide with the carrot disk at the end, there were now more strands growing towards the carrot than away from it. Although no infection had yet taken place, vigorous mycelial growth was present on the surface of the carrot pieces. The effect of the carrot pieces on growth towards them was more striking than the figures for *mean* number and thickness of strands suggest, as there were now strands up to $80\,\mu$ across stretching from the inoculum to the carrot surface, whereas no strand more than $40\,\mu$ across was observed elsewhere. It appears that once the leading hyphae touched the carrot surface they were able to transmit back some impulse which initiated vigorous strand development along them, the original strands becoming thicker than they would otherwise have been, and younger strands developing along other hyphae which, presumably, would not otherwise have become thus involved. The latter were young and narrow at the time measurements were made and, because of this, the mean thickness of the strands growing towards the carrot was much the same as that of these growing away from or formed in the absence of carrot. It seems possible that, had smaller inoculum disks been used, the increased growth towards and on to the carrot pieces would have been made at the expense of growth in the opposite direction.

It is clear that, in this case at least, any attraction for the hyphae, if it existed, must have been exerted over a short distance only. Nevertheless, it is obvious that, once the hyphae made contact with the root, mycelial growth was greatly stimulated. It may be, however, that actively growing carrot roots can exert an attraction over some distance.

Effect of various soil conditions. Garrett (1946) found that strand growth over the surface of potato tubers from agar inocula was greater, using Harpenden allotment soil, in a 1 soil:3 sand mixture than in whole soil; soil acidity (pH 5·1) depressed growth in whole soil at 40 and 60 %, but not at 80 % m.h.c. and not in the soil:sand mixture. Otherwise there were no real differences between growth at the three moisture levels. This adverse effect of soil acidity was also shown in the growth of mycelium over Rossi-Cholodny slides buried in soil. Garrett attributed this poorer growth to the joint action of three factors—acidity, low soil moisture, and less than optimum soil texture. Apart from this there is little experimental information to serve as a guide except for Whitney's (1954) experiments with carrots, which showed that the disease developed satisfactorily in soils of pH 3·5–7·9 and that a soil temperature of 20° C. was the optimum for disease development in this host.

Experiment 1. The effect of various soil conditions, as set out in Table 6, was examined, the experimental procedure being the same as in the experiment on the effect of a food base. Soils were brought to 60 % m.h.c., except where otherwise indicated, and the soil:sand mixture again consisted of 1 vol. soil:3 vol. sand. To investigate the effect of soil texture, apart from dilution with sand, Botanic Garden soil was sieved to provide samples of particle size 1·5–2 (coarse), 1–1·5 (medium), and < 1 mm. (fine). Denitrified soil was prepared by mixing it with an excess of wheat straw and keeping it moist in a warm glasshouse for 6 weeks, the soil then being air dried and the straw sieved out. The Kettering loam:sand, which had an original pH of 4·9, was made alkaline by the addition of powdered calcium carbonate and kept moist in a warm glasshouse for 6 weeks before use. The denitrified soil and Kettering loam, which might have had a particle size distribution different to that in untreated Botanic Garden soil, were also sieved to give samples of particle size 1–1·5 mm., so that the likelihood of any differences between growth in these soils being caused by differences in soil texture would be reduced. Grassmeal and peameal were added at the rate of 1 % of dry weight. The pH of each soil was measured at the end of the experiment, but it is not thought that any of the pH changes which occurred is likely to have influenced the results.

Table 6. *Effect of various soil conditions on growth and mycelial strand production by* Helicobasidium purpureum

	Growth after 17 days*			Growth after 35 days*			
	Extent (mm.)	No. of strands	Thickness of strands (μ)	Extent (mm.)	No. of strands	Thickness of strands (μ)	Final pH of soil
tt. loam:sand (pH 4·9)	27	2·3	23	55	4·8	26	4·9
tt. loam:sand (pH 7·5)	28	2·8	25	69	4·5	28	7·5
tt. loam	22	2·5	22	51	5·7	26	4·7
. Gard. soil:sand	23	1·0	27	53	3·8	24	7·5
. Gard. soil, 20 % m.h.c.	20	0·8	22	64	3·5	26	7·6
. Gard. soil, 40 % m.h.c.	33	3·7	27	74	5·5	28	7·5
. Gard. soil, 60 % mh.c.	32	3·0	27	72	5·8	29	7·5
. Gard. soil, 68 % m.h.c.	28	4·5	27	69	6·3	28	7·6
. Gard. soil, coarse	24	2·5	26	60	4·0	35	7·4
. Gard. soil, medium	29	3·5	23	62	6·3	24	7·4
. Gard. soil, fine	30	3·8	21	69	6·8	27	7·4
. Gard. soil + grassmeal	32	6·2	28	78	7·2	30	7·8
. Gard. soil + peameal	25	1·7	25	70	6·7	27	7·4
. Gard. soil, denitrified	35	8·0	27	74	9·8	28	7·4

* Mean of six replicates.

Table 6 shows little difference in amount of growth in Kettering loam: sand at pH 4·9 and 7·5. Growth in the acid Kettering loam of medium texture was only slightly less than in Botanic Garden soil of the same texture. Variation in moisture content in Botanic Garden soil produced no striking differences, except that growth at 20 % m.h.c. was a little less than at other moisture levels. This, however, may have been caused by the mixing in of the water by stirring in this instance, as an equal distribution could not be obtained by simple addition. Variation in texture produced some effect in that less strands were produced in the coarse soil than in the medium and fine ones. They were thicker in the coarse soil, however, so the amount of growth was much the same.

Growth and strand production were greater in denitrified soil (particle size 1–1·5 mm.) than in untreated soil of the same texture. This effect was not prevented when the denitrified soil was moistened initially with solutions of sodium nitrate to give both 5 and 12·5 mg. of nitrogen/100 g. soil, and the reason for the phenomenon remains unknown.

The addition of grassmeal caused a slight improvement in growth, and the addition of peameal caused an initial check, the effects of which were disappearing by the time the first reading was made. In a preliminary experiment in which these organic supplements were added to a soil:sand mixture there was little growth after 17 days but after 35 days vigorous growth had taken place, peameal causing the biggest check in this instance also.

Addition of sodium nitrate, ammonium sulphate or 'Casamino Acids', to give either 5·0 or 12·5 mg. nitrogen/100 g. soil, had no effect on amount of growth (data not given in Table 6).

The most obvious and consistent effect observed in Garrett's (1946) experiments, i.e. the substantial increase in extent of mycelial growth produced by diluting the soil with sand, was not reproduced in these experiments. If this effect were due to improved soil aeration, this discrepancy is explicable, because aeration between the soil surface and the appressed glass slide would be better than at the surface of slides buried in soil, as employed by Garrett.

Morphology and development of mycelial strands

The mycelial strands of *H. purpureum* are composed of aggregates of hyphae, 2·5–7·0 μ in diameter with septa 55–200 μ apart, such as have been described by Duggar (1915), Faris (1921) and Buddin & Wakefield (1924). These strands may be up to 1 mm. in diameter, though usually much less, branching and becoming thinner with increasing distance from the food base; the apical regions are composed of individual branching hyphae extending for some distance ahead of the zone of aggregation. On roots the pattern of growth of the strands frequently appears as a confused network, as Faris (1921) noted on potato tubers. This is because tubers and roots usually become infected in patches and strands subsequently develop radially from each area of infection, the directional effect becoming obscured in areas into which the mycelium is growing from different directions. The principal of growth away from the food base, however, remains.

Apart from describing the strands as loose aggregates of hyphae, previous workers have given few details of their structure and method of formation. Garrett (1956*a*) noted that the strands appeared to grow in thickness by continued accretion of fresh hyphae, coming from the substrate, to the base of the strand, and frequent anastomoses could be found between the hyphae composing the strands.

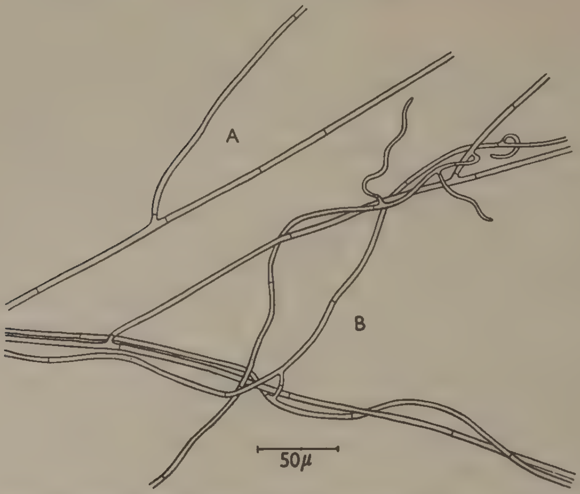
Boedijn & Steinmann (1931) noticed that *H. compactum* produced simple strands on host roots, and Ito (1949) reported that the strands of *H. mompa* were 0·1–1·2 mm. in diameter with no noticeable differentiation, being only an aggregation of numerous entangled hyphae. Tanaka (1891), however, in his original description of *H. mompa*, stated that the thickening of a strand was affected either by the copious branching of a single hypha, or by the coalescence of two or more strands. He figured a portion of a strand showing the loosely interwoven hyphae running more or less parallel. Some branching is present as also is an anastomosis, and the picture is remarkably similar to that of a portion of a strand of *H. purpureum* (Pl. 15; Text-fig. 2, B, C). He also figured a cross-section of a strand showing it to be flattened and to have the largest hyphae towards the centre. Strands of *H. purpureum* from the surfaces of roots or glass slides were also flattened, but this was apparently only a positional effect, as in instances where they were induced to form away from continuous surfaces, they were approximately circular in cross-section, again with the youngest and thinnest hyphae towards the outside.

A tendency to strand formation was observed on the glass walls of tubes and other vessels containing old cultures, and once in the aerial mycelium of a group of old cultures on meat-malt agar, at about the time sclerotia had commenced to develop. However, no method was found of producing strands at will in pure culture, even though the fungus was grown under a very wide range of physical and nutritional conditions. Hence strand development was studied on the undersurfaces of slides pressed firmly into Petri dishes of moist soil.

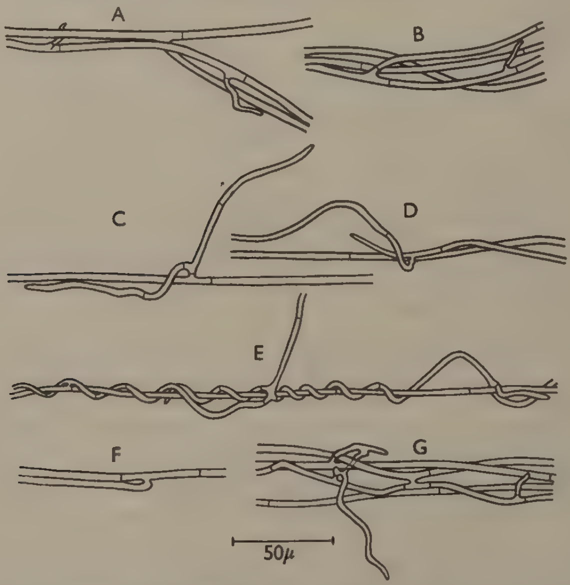
At first a sparse growth of robust hyphae (Text-fig. 1 A), branching periodically in the direction of growth, grew out from the food base in a radiating fashion. Once a system of leading hyphae was established, other hyphae subsequently growing out from the food base very soon came across one of these leading hyphae or one of their branches, and tended to follow them (Text-fig. 1 B). A hypha growing out from the food base, making contact with and following a leading hypha, frequently accumulated following hyphae of its own in the same way, which became added to the main strand at the point of contact (Pl. 15, fig. 2). Thus accumulation of hyphae and small strands by a leading hypha was responsible for the development of a main strand, increasing as it did in thickness from the base, which sometimes had an appearance resembling that of a river delta. A general loose interweaving served to bind the hyphae of the strand together, pronounced twining (Text-fig. 2 E) being uncommon, as also did the frequent anastomoses (Text-figs. 1 B; 2 G).

Growth of branches of the following hyphae was usually much restricted and they curled round the other hyphae in the strand (Text-fig. 2 A, G), further serving to bind the hyphae and often becoming involved in anastomoses with other branches (Text-fig. 2 G). Very complicated configurations of short branches and anastomoses occurred at some points. Branching nevertheless, did play a recognizable part in increasing the thickness of strands, growth usually being in the direction of strand growth (Text-fig. 2 B), but sometimes backwards (Text-fig. 2 C). A hypha growing back towards the food base often anastomosed with one growing the other way (Text-fig. 2 D). Branching seemed to be more important in the distal portion of a strand; the portion of a strand of *H. mompa* illustrated by Tanaka (1891) also appears to be a distal portion.

Strands and leading hyphae also accumulated other hyphae which



Text-fig. 1. Mycelial strand development in *H. purpureum*. The general direction of growth is from left to right. For explanation see text.



Text-fig. 2. Mycelial strand development in *H. purpureum*. The general direction of growth is from left to right. For explanation see text.

made contact at various points along their length. Such hyphae usually proceeded in the general direction of growth after making contact, but sometimes grew back towards the food base or sent branches in both directions (Text-fig. 2E). Hyphae growing backwards or forwards along others sometimes anastomosed at the tip and ceased growing (Text-fig. 2D, F). By following such hyphae back for distances greater than shown in the drawings, it was evident that they were originally separate hyphae and not, as might be supposed from a casual inspection of Text-fig. 2D, F, merely a parent hypha with its branch.

A leading hypha sometimes accumulated following hyphae at various points along its length and the strand, initially, consisted of a number of aggregations connected by only one or very few hyphae. Subsequently growth and anastomosis, however, gave longitudinal continuity.

The branching of a strand was usually caused by branching of the original leading hypha, some of the following hyphae diverging and following the branch rather than the parent hypha (Pl. 15, fig. 3; Text-figs. 1B; 5A). Less commonly branching was caused by the divergence of a following hypha from a leading hypha or strand, the subsequent following hyphae becoming divided as before. Branching was also caused occasionally by one leading hypha crossing another, and some of the hyphae following one leader diverging and following the other at the intersection.

Where a leading hypha met and followed a strand or another leading hypha, a reticulate system developed, and a branch from one strand sometimes grew across and became part of another. However, as Garrett (1956*a*) noted for *Psalliota hortensis*, this was more common near the food base, where the density of hyphae increased the chance of such contacts.

Strands were occasionally observed to disintegrate into their constituent individual hyphae, though this was usually where there was some mechanical block in the soil preventing further extension of the hyphae in their original aggregated state.

As a result of the processes described above, strands were built up which were thickest at the base, becoming thinner with increasing distance from the food base, largely as a result of branching, which more than counteracted any increase in thickness that was caused by branching of hyphae within a strand or accumulation of unrelated hyphae. Because of this habit of following, hyphae not involved in strands were relatively few, although the branching leading hyphae retained their individuality for a considerable distance ahead of any following hyphae.

It seems possible that the restriction of development of most of the branches of the following hyphae enabled their apices to make greater, and perhaps faster, longitudinal growth, the strand being thus given greater uniformity and longitudinal continuity. Continuity, however, is also ensured by the frequent anastomoses. Nothing is known of the stimulus that induced one hypha to follow another, but it appeared that the smaller hyphae tended to follow the larger ones, and single hyphae to follow strands when they encountered them.

SURVIVAL OF SCLEROTIA IN THE SOIL

The sclerotia of *H. purpureum* have been described by Duggar (1915), Faris (1921), Hull & Wilson (1946) and Garrett (1946), it being assumed that they are responsible for prolonged survival of the fungus in the soil in absence of host roots. In a study of the disease in sugar beet, Hull & Wilson (1946) observed that the sclerotia appeared to develop from a well-nourished mycelium at an advanced stage of attack and were not found at all in connexion with light or early infections. They may be produced on the mycelial web or strands, as described by Duggar (1915) and Faris (1921), but, as Garrett (1946) pointed out, on sugar beet and carrot they are most frequently formed on small lateral roots, along which they may even be strung like beads on a thread (Pl. 16, fig. 1).

Sclerotia formed under natural conditions are very variable in size and those examined in this study were largely composed of a whitish pseudoparenchyma of swollen, thin-walled cells. These cells were often irregularly shaped, as figured by Duggar (1915), and were usually 10–15 μ or occasionally up to 30 μ in diameter. The pseudoparenchyma was surrounded by a 'rind' of dark, thick-walled cells, which is clothed with a persistent velvety felt of brown hyphae.

Sclerotia have been observed in cultures by Buddin & Wakefield (1924), Garrett (1946) Whitney (1954) and in the present investigation. Similar bodies have been described in cultures of *H. mompa* by Ito (1949). Buddin & Wakefield (1924) observed that those formed by *H. purpureum* were composed of more compact and regular hyphae than the natural ones described by Duggar (1915). Those formed on malt agar in the present study were composed of a mass of interwoven, swollen and much branched hyphae, which appeared to arise as branches of hyphae of normal thickness. Sections of these sclerotia appeared very similar to sections of natural sclerotia, the cells, usually 8–12 μ in diameter, being a little smaller and more regular than those of the natural sclerotia. However, while they were clothed in a velvety felt similar to that occurring on natural sclerotia, there was no evidence of any well defined 'rind'. After a period of burial in soil, however, the outer layers of these sclerotia were found to have darkened and become more compact. These bodies have been observed to be more abundant on media of relatively high carbon/nitrogen ratio and colonies grown on such media survive in soil very much better than those on media of lower carbon/nitrogen ratio (Garrett, 1946).

Nothing is known of the factors affecting sclerotial survival in soil and the experiments to be described hereunder were therefore set up.

Methods

Garrett (1949) used agar colonies, ten per 9 cm. Petri dish, for his experiments on survival in a soil:sand mixture (1 vol. Harpenden allotment soil:3 vol. sand); he tested the viability of each colony by burying it in the middle of a group of four stump-rooted carrots growing in a seed box; the carrots were examined for infection 2 months later. The production and testing of large numbers of colonies by this method is slow, and an attempt was therefore made to produce a less laborious method.

Dunlap (1941) devised a convenient method for obtaining sclerotia of *Phymatotrichium omnivorum* by inoculating autoclaved flasks containing layers of various seeds lying on top of moist soil. The fungus produced abundant sclerotia on strands which grew into the soil. This was tried for *H. purpureum* using rye, turnip, red clover and vetch seed, but there was no strong mycelial growth into the soil. Nevertheless, sclerotia of the type observed in agar cultures developed on the colonized seeds; rye grain proved to be the most suitable substrate. An important advantage of rye grain over agar as a substrate for the production of sclerotia was that some hundreds of individual colonies with sclerotia could be produced by inoculation of a single flask, whereas each agar colony had to be produced by a separate inoculation. The cultures were prevented from cohering into a mass of mycelium by admixture with a suitable volume of sand. Thus 100 g. sand and 5 g. rye grain (soaked overnight in water) were mixed dry and then 15 ml. water were added to the mixture in a 250 ml. flask; such flasks were autoclaved at 15 lb. pressure for 1 hr. and inoculated with isolate B. The flasks were incubated at 22·5° C., shaken at intervals to keep the grains separate, and capped after growth had got started during the first fortnight, with moisture-proof Cellophane. After 10 weeks incubation of the flasks, the grains appeared as shown in Pl. 16, fig. 2; microscopical examination showed that the structure of the sclerotia that had developed on the grains was very similar to that of those formed on agar media.

There still remained, however, the problem of finding a more manageable method of testing the viability of large numbers of such colonies. Owing to the slow growth of *H. purpureum*, it was unsatisfactory to test viability by surface-sterilizing and plating-out on agar, because the plates became overgrown with other fungi. The following new method of testing viability of sclerotia on rye grain (and other substrates) was therefore tried and proved satisfactory; the colonies or individual sclerotia to be tested were placed in contact with segments of carrot root and potato tuber in Petri dishes of moist sand. Surprisingly few of the root or tuber pieces rotted during the course of 1 month and, at the end of this period, *H. purpureum* could be observed growing over the root or tuber surfaces from the sclerotia; infection cushions sometimes developed before the end of the incubation period. The results with carrot root were more satisfactory than those with potato tuber, as moulds were apt to develop on the surface of the latter and growth of *H. purpureum* was difficult to discern. A technique was finally devised in which two transverse slices of carrot root, approximately 1·5 cm. thick, were placed in a dish containing 50 g. sand brought to 100% m.h.c., and two colonized rye grains to be tested for viability of *H. purpureum* were placed on opposite sides of each carrot piece. The dishes were incubated at 22·5° C., in batches of twelve, contained in polythene bags to prevent evaporation; growth and infection by *H. purpureum* from the inocula were sufficiently advanced after 4–5 weeks to enable viability counts to be made. Very few carrot disks rotted during this period so long as the carrots were mature, lifted and washed carefully, and sliced with a clean razor blade. Under these circumstances the cut surfaces rapidly calloused over during incubation.

Having devised satisfactory techniques for the production and viability testing of large numbers of sclerotia, it then became possible to set up experiments to ascertain the effect of soil conditions on survival of sclerotia. Jam jars (1 lb.) were used as soil containers; soils were prepared and moisture holding capacities determined and maintained as described by Garrett (1956*a*). In each jar fifteen grains colonized by *H. purpureum* were placed with 5 cm. soil above and below them; the jars were kept on a laboratory bench and the soils maintained at 50% m.h.c., except where otherwise indicated. For viability tests three jars of each experimental series were taken, giving forty-five grains in all; the colonized grains were washed free of soil on a sieve.

Experiments

Experiment 1. Colonized grains after 10 weeks incubation in the flasks were buried in Kettering loam and Kettering loam:sand (1:3) with and without the addition of 1% peameal and 1% grassmeal. Maximum and mean maximum air temperatures* in the laboratory during the experimental period were 26° C. and 21° C., respectively, and the minimum and mean minimum 11° and 15° C. Grains were tested after 10 and again after 20 weeks. (Table 7).

As can be seen, survival was poor in these very acid soils.

Table 7. *Sclerotial survival in Kettering loam*

	Viability %		
	After 10 weeks	After 20 weeks	Final pH of soil
Kett. loam	40	12	4·5
Kett. loam + 1% grassmeal	24	26	4·5
Kett. loam + 1% peameal	37	16	4·3
Kett. loam:sand	30	23	4·9
Kett. loam:sand + 1% grassmeal	58	46	5·0
Kett. loam:sand + 1% peameal	44	21	4·0

Experiment 2. As a result of the poor survival of *H. purpureum* in Expt. 1, it was decided to investigate the effect of age of the colonized grains at time of burial in Botanic Garden soil:sand (1:3, pH 7·5); this soil was similar to that used by Garrett (1949) in his survival studies. The maximum and mean maximum temperatures during the experimental period were 29° and 21° C., respectively, and the minimum and mean minimum 8° and 14° C. (Table 8).

Table 8. *Effect of age of grain colonies at burial on sclerotial survival in Botanic Garden soil:sand mixture*

	Viability (%)		
Age of colonies at burial	After 6 weeks	After 12 weeks	After 18 weeks
8 weeks	79	42	44
10 weeks	75	71	72
12 weeks	70	79	64

It is clear that the survival of grain colonies was good when they were 10–12 weeks old at time of burial. The poorer survival of those aged 8 weeks at time of burial was no doubt because they had developed fewer mature sclerotia.

Experiment 3. To check the reliability of the viability test and to observe the survival of natural sclerotia, a limited number of such sclerotia, which had been obtained from infected carrots, were buried in Botanic Garden soil:sand. The maximum and mean maximum air temperatures during the experimental period were 29° and 21° C., respectively, and the minimum and mean minimum 8° and 14° C.

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* Determined from a record of *daily* readings of a maximum-minimum thermometer.

The viability of the sclerotia when collected was 86 % and after 18 weeks in jars of Botanic Garden soil:sand, 65 %.

Experiment 4. This experiment was set up to compare survival of 10-week-old rye grain colonies in Kettering loam:sand and Botanic Garden soil:sand with treatments as shown in Table 9. The denitrified soil:sand was prepared as before, grassmeal and peameal were added to the soil at the rate of 1 % dry weight and nitrogen sources at the rate of 12·5 mg. nitrogen/100 g. air-dry soil. As it was thought that sclerotia might undergo further development and maturation when buried in soil, two series were buried in Botanic Garden soil:sand for 6 weeks and then reburied as shown in Table 9. A series was also set up in a shelter out of doors where much lower temperatures prevailed, and in another series the moisture content was maintained at 80 % m.h.c. instead of at 50 %. The maximum and mean maximum temperatures in the laboratory during the experimental period were 29° and 21° C. respectively, and the minimum and mean minimum 8° and 16° C. (Table 9).

Table 9. *Sclerotial survival under various soil conditions*

	Viability (%)			pH of soil		
	After 6 weeks	After 12 weeks	After 18 weeks	After 6 weeks	After 12 weeks	After 18 weeks
Kett. loam:sand	26	8	10	5·5	5·4	5·3
Bot. Gard. soil:sand	75	71	72	7·5	7·6	7·5
Bot. Gard. soil:sand, denitrified	79	80	66	7·4	7·6	7·5
Bot. Gard. soil:sand + $NaNO_3$	85	82	69	6·9	7·3	7·4
Bot. Gard. soil:sand + $(NH_4)_2SO_4$	83	66	71	7·0	7·0	7·1
Bot. Gard. soil:sand + Casamino Acids	81	76	88	6·9	7·0	7·0
Bot. Gard. soil:sand + peameal	82	75	77	6·2	6·4	6·4
Bot. Gard. soil:sand + grassmeal	79	82	79	6·9	6·9	7·0
Bot. Gard. soil:sand, cold*	76	82	87	7·4	7·4	7·6
Bot. Gard. soil:sand, 80 % m.h.c.	80	72	76	7·3	7·3	7·3
Kett. loam:sand, reburied†	—	73	29	—	5·0	4·9
Bot. gard. soil:sand, reburied†	—	80	75	—	7·5	7·5

* Kept out of doors (max. and mean max. temperatures, 25° and 11° C.; min. and mean min., −11° and 1° C.)

† Buried for the first 6 weeks in Botanic Garden soil:sand and then reburied as shown.

Survival of the sclerotial colonies in Botanic Garden soil:sand appears to have been unaffected during the experimental period by a very wide range of conditions. The pH measurements show that there were changes in pH, though not large ones, in some series during the experiment, but these were not associated with any differences in viability of the sclerotial colonies. As can be seen, survival was good in Botanic Garden soil:sand but very poor in Kettering loam:sand. Burial of the colonies in Botanic Garden soil:sand for 6 weeks, previous to burial in Kettering loam:sand, reduced but did not prevent this effect.

When the colonized grains were washed out of the soils, a considerable amount of soil was observed adhering to those that had been buried in Kettering loam:sand, whereas this occurrence was rare amongst those that had been buried in Botanic Garden soil:sand (Pl. 16, fig. 3). This had been noticed in Expt. 1, but little attention was paid to it at the time. Microscopical examination now showed that the soil was enmeshed in a dense growth of the easily recognized hyphae of *H. purpureum*, and it is assumed that the fall in viability was caused, at least in part, by spontaneous germination of the sclerotia.

Apart from this, the main conclusion to be drawn from this experiment is that very wide changes in the available nitrogen content of soil did not affect the survival of sclerotia, although Garrett (1949) had observed that the nitrogen content of the substrate appeared to exercise a controlling effect on their original formation.

Experiment 5. Colonies on 6 % malt agar plates, ten colonies per plate, were grown as described by Garrett (1949), cut out and then buried under a variety of soil conditions when they were 8 weeks old, at which stage they bore numerous sclerotia. The colonies were buried in the same way as the rye-grain colonies, fifteen per 1 lb. jam jar; soils and treatments and results are set out in Table 10, details being as described for Exp. 4.

Table 10. *Survival of agar colonies bearing sclerotia under various soil conditions*

	Viability (%)			pH of soil		
	After 6 weeks	After 12 weeks	After 18 weeks	After 6 weeks	After 12 weeks	After 18 weeks
Kett. loam:sand	82	58	42	5·2	5·1	5·1
Bot. Gard. soil:sand	91	77	89	7·3	7·4	7·5
Bot Gard. soil	97	91	83	7·4	7·4	7·5
Bot. Gard. soil, denitrified	93	80	78	7·5	7·6	7·5
Bot. Gard. soil + $NaNO_3$ (12·5 mg. N/100 g. soil)	97	90	82	7·4	7·3	7·4
Bot. Gard. soil + $NaNO_3$ (6·25 mg. N/100 g. soil)	95	79	75	7·3	7·2	7·4
Bot. Gard. soil + 1 % peameal	100	84	74	6·9	6·9	7·0
Bot. Gard. soil + 1 % grassmeal	96	86	80	7·4	7·3	7·4

The figures in Table 10 show no marked difference in survival between the various treatments in Botanic Garden soil or between these and Botanic Garden soil:sand. Survival in Kettering loam:sand was again poor. Survival periods for sclerotia on agar colonies compared closely with those for sclerotia on grain colonies (Table 9), so that it is legitimate to compare results obtained by Garrett with those reported here.

Experiment 6. In order to see whether or not low soil pH was responsible for the spontaneous germination and fall in viability of sclerotia, 10-weeks-old rye grain colonies were buried, as before, in both unaltered Kettering loam:sand and Kettering loam:sand which had been made alkaline as described earlier for the experiments on growth of mycelial strands. Another series was set up in which the colonies were buried in Botanic Garden soil:sand for 6 weeks prior to reburial in the Kettering loam:sand.

The maximum and mean maximum air temperatures during the experimental period were 29° and 22° C., respectively, and the minimum and mean minimum 8° and 14° C. (Table 11).

Table 11. *Effect of* pH *on sclerotial survival in Kettering loam: sand mixture*

	Viability (%)			Final pH of soil
	After 6 weeks	After 12 weeks	After 18 weeks	
Acid soil	44	57	20	5·1
Alkaline soil	78	67	77	7·3
Acid soil, reburied*	—	68	63	4·7
Alkaline soil, reburied*	—	83	84	7·3

* Buried for the first 6 weeks in Botanic Garden soil:sand (pH 7·5) and then reburied as shown.

The fall in viability in the acid soil was much greater than that in the alkaline; observations made when the grain colonies were washed from the soil again showed this to be associated with spontaneous germination of the sclerotia. Survival in the acid soil was improved when the colonies had been buried previously in Botanic Garden soil:sand. This may have been the result of the persistence of alkaline conditions in and around the colonies for some time after their burial in acid soil, rather than of any further development and maturation of the sclerotia. Nevertheless, it is clear that in this soil, pH is an important factor in determining the percentage of spontaneous germination and, conversely, length of survival of the colonies.

In Botanic Garden soil:sand the only stimulus which has been observed to cause vigorous germination of sclerotia is contact with a carrot root. Where sclerotia were placed 0·5 cm. away from pieces of carrot root there was no germination, indicating that, under the conditions of the experiment, the stimulus could not operate over such a distance. Neither was any germination observed when the soil was moistened with carrot root exudate instead of water. This is in keeping with the observations made in the experiments on the effect of carrot roots on mycelial growth. It may be, however, that where actively growing roots are concerned, the sclerotia can be stimulated to germinate without being in absolute contact with the roots.

General conclusions concerning survival of Helicobasidium purpureum *as sclerotia*

In these experiments with sclerotia, with the one exception of the acid Kettering loam:sand mixture, survival was unaffected by a wide range of soil conditions. Heavy additions of organic matter did not reduce longevity, though they have been reported to hasten disappearance of sclerotia of *Phymatotrichum omnivorum* (Mitchell, Hooton & Clark, 1941; Clark, 1942; Rogers, 1942) and *Sclerotium rolfsii* (Johnson, 1953). Neither did a high soil moisture content and consequent poor aeration have any adverse effect, such as has been observed for *Phymatotrichum omnivorum* (King, Loomis & Hope, 1931; King & Eaton, 1934), *Sclerotinia sclerotiorum* (Stoner & Moore, 1953), *S. trifoliorum* (Christoff, 1941–2) and *Sclerotium rolfsii* (Fajardo & Mendoza, 1935; Chowdhury, 1945). It is possible, however, that some differences in survival might have been detected between the various experimental series, if the survival tests had been prolonged beyond 18 weeks.

It is interesting that the only instance of poor survival of the sclerotia of *H. purpureum* was associated with a high incidence of spontaneous germination. A similar association has been observed in instances where the viability of sclerotia of *Phymatotrichum omnivorum* was reduced (King & Eaton, 1934; Mitchell *et al.* 1941); similarly, the sclerotia of *Sclerotium rolfsii* have been reported to germinate spontaneously at least on the surface of unsterilized soil (Fajardo & Mendoza, 1935; Leach & Davey, 1938; Chowdhury, 1945). An analogous case is the spontaneous germination of conidia of *Helminthosporium sativum* in soil, in response to the addition of soya-bean meal and the subsequent death of the germ tubes (Chinn, 1953). It seems probable that death of the germ tubes results from their failure to colonize the substrate in competition with other micro-organisms.

Garrett (1956*a*) has pointed out that such a liability to spontaneous germination in the absence of a host root stimulus must be regarded as a serious defect in the functional efficiency of sclerotia, and he cites instances, for example, that of the sclerotia of *Phymatotrichum omnivorum*, where this functional weakness has been exploited for purposes of disease control. It is possible that acidification of the soil would bring about the same result in the case of *H. purpureum*, but further studies are necessary with other soils before the importance of acidity can be fully assessed.

One other feature of these results, although negative, deserves comment. Many of the experimental treatments were designed to test the possibility that the available nitrogen content of the soil might determine survival of sclerotia, either by affecting a possible further process of maturation when sclerotia that had developed in pure culture were placed in soil, or in some other way. This hypothesis seemed reasonable in view of the fact that nitrogen level had been earlier found to control the formation of viable sclerotia on agar media. In view of the wide range of conditions with respect to nitrogen tested in the foregoing experiments, however, it seems safe to assert that the survival of sclerotia, once mature, is unlikely to be affected either by a deficiency or by a non-toxic excess of available nitrogen. These experiments may thus be deemed to have answered one of the chief questions posed by the earlier study of Garrett (1949) on survival of sclerotia in *H. purpureum.*

In nature, the ability of the fungus to survive in the absence of host roots depends, initially, on its ability to form sclerotia on host roots, and further investigation of this aspect of the life history of *H. purpureum* is desirable. However, the ability of *H. purpureum* to make satisfactory growth and of its sclerotia to survive in soil under a wide range of conditions, as demonstrated in this study, sufficiently explains its wide distribution as a root-inhabiting fungus.

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EXPLANATION OF PLATES 15 AND 16

Plate 15

Mycelial strands of *H. purpureum*. The general direction of growth is from left to right.

Fig. 1. Diverging strands (× 200).
Fig. 2. Coalescence of strands (× 200).
Fig. 3. Origin of a strand branch (× 500).

Plate 16

Fig. 1. Sclerotia from naturally infected carrots (× 1½).
Fig. 2. Rye grains colonized by *H. purpureum* and bearing sclerotia (× 1½).
Fig. 3. Colonized rye grains as they appear when washed out of soil for a viability test (× 1½). Those in the upper row are from a Kettering loam: sand mixture, those in the lower from a Botanic Garden soil: sand mixture. For explanation see text.

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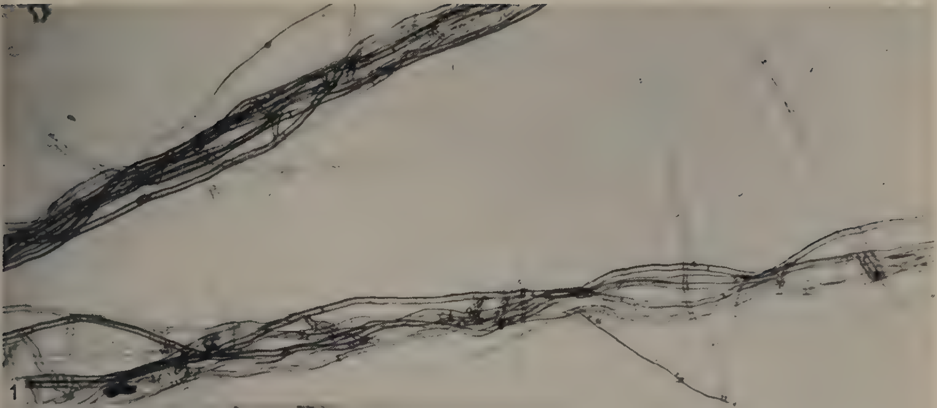

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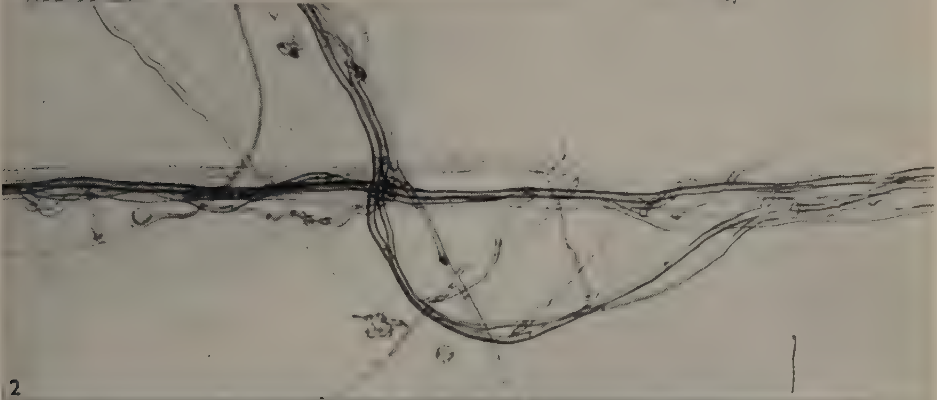

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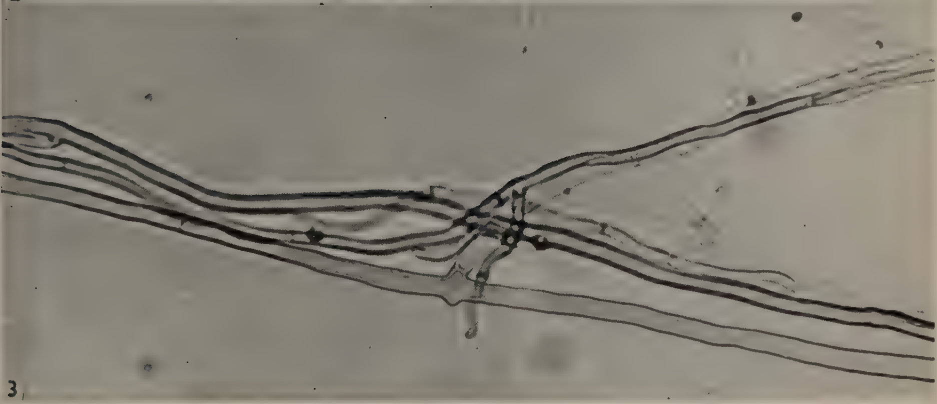

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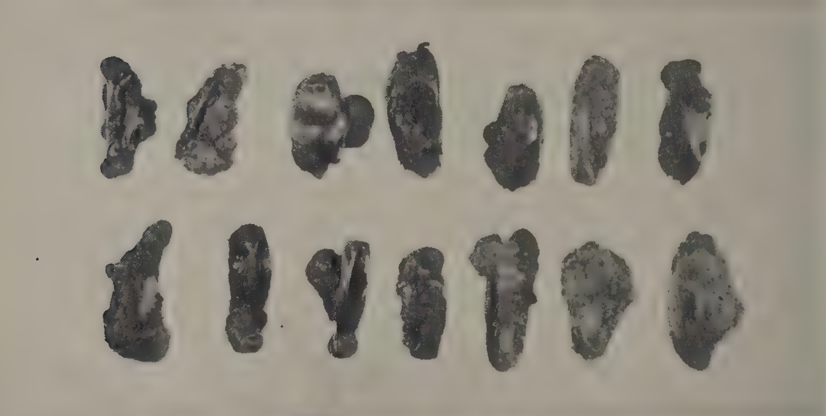

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Trans. Brit. mycol. Soc. **41** (3), 309–319 (1958).

STUDIES ON SOIL CHYTRIDS

III. ON *KARLINGIA ROSEA* JOHANSON AND A MULTI-OPERCULATE CHYTRID PARASITIC ON *MUCOR*

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(With Plate 17 and 4 Text-figures)

From a natural population of *Karlingia rosea* Johanson in which several different types of development from the zoospore were represented, nineteen separate monosporangial cultures were made. Potassium tellurite was found to be an effective bactericide in this work. The mode of development in monocentric eucarpic chytrids is of interest as it has previously been proposed that this should be used as a major criterion in classification. All the cultures proved to be of a single type, in which there was even expansion of the zoospore cyst, and this characteristic remained constant through several generations. The theory is expressed that there may be several distinct lines of descent in this species, each different in regard to initial development. Despite slight differences in regard to pigmentation and morphology, the culture material generally agreed well with Johanson's account. Further thalli which could not be cultured were also obtained. These were considered to be specimens of *K. rosea*, but of a type not previously described, with the sporangium ornamented with short setae.

A multi-operculate chytrid parasitic on *Mucor* is also described.

KARLINGIA ROSEA JOHANSON]

When soil from Osterley Park, Middlesex, was baited with P.T. 300 non-waterproof cellophane, chytrid thalli which agreed with Johanson's (1944) description of *Karlingia rosea* were occasionally obtained. To obtain the organism in quantity commercial waterproof cellophane (Diophane) was used. This material is particularly suitable as a bait since it floats in the Petri dishes of soil (Willoughby, 1956). The cellophane was gently boiled on the surface of distilled water before use. Vigorous boiling or autoclaving removes the waterproofing lacquer and the floating property is lost. Following incubation at 20° C., microscopic examination revealed that only the cut edges of the pieces of cellophane, and the places on the lower surface where the waterproofing layer had been disrupted by boiling, were available for chytrid growth; but a large number of sporangia usually appeared in these positions.

In addition to the thalli which had developed as a result of even expansion of the zoospore and were quite superficial, as reported by Johanson, others were present in which a part or the whole of the original zoospore cyst remained unexpanded (Text-figs. 2*w*; 3*a–d*, *e*, *i*), and in these cases the thalli were often embedded in the cellophane (Pl. 1*d*). Thalli of the latter type have previously been reported for *K. rosea* by Karling (1947). Despite differences in development of the sporangia, the dehiscence mechanism and zoospore morphology appeared identical in all cases in my material. Particular interest is attached to the mode of thallus development from the zoospore, as Whiffen (1944) has proposed that this should be used as a major criterion in classification. She divided the monocentric eucarpic chytrids into two families, the Rhizidiaceae and the Entophlyctaceae. In the former group the zoospore swells to become a sporangium, while in the latter the encysted zoospore gives rise to a germ tube which enlarges into a zoosporangium or prosporangium. When cultures were made from my material, it was found, rather unexpectedly, that the only type of development which could be obtained was that in which the zoospore expanded evenly to form the thallus, and in view of the importance attached to development by Whiffen, it was felt that further study was necessary. A detailed investigation involving the study of unisporangial isolations was therefore undertaken, with the sole object of determining the consistency of development in *K. rosea*. It appeared that it would be interesting to know whether various types of development are represented within a series of sporangia descended from a single spore over a number of generations, in order to determine whether or not any single zoospore has the potentiality to develop in more than one way.

Accordingly, the pieces of cellophane from the initial platings were vigorously washed with sterile charcoal water and then placed in a small amount of the same liquid and warmed by means of an electric lamp. This induced dehiscence in many sporangia, and enormous numbers of zoospores were available for streaking out on to agar. The agar was quarter strength Emerson's YpSs, in 2 % plain agar (see Crasemann, 1954), and made up with soil-extract solution as recommended by Emerson. It was poured on to slides supported on glass rods in Petri dishes, with water added to keep the chamber moist, all of these materials being previously sterilized. No attempt was made to determine from which sporangia the zoospores emanated, but in view of the results it appears that during this experimental work, zoospores were never obtained from sporangia which had developed as a swelling on the germ tube formed by the zoospore. For some reason, sporangia of this type could not easily be induced to dehisce. Zoospore streaks made on to the agar (pH 7·2–7·4) normally soon became overrun by bacteria, but by using 0·01 % potassium tellurite autoclaved in the medium, growth of most of these was suppressed and it was possible to cut out individual developing thalli with a small block of the surrounding agar after 2 or even 3 days (Pl. 17*a*). These small blocks were transferred to McCartney bottles to establish cultures. Each culture was derived from a single sporangium, and therefore in effect represented a monospore isolation. All cultures were incubated at 20° C. The whole procedure was repeated on a number of different occasions to ensure that the isolations were made from zoospores derived from a number of different sporangia. The bottles used contained 5 ml. of soil extract solution, or 5 ml. of quarter strength Emerson's YpSs solution. 0·01 % potassium tellurite was included with both of these two media used. P.T. 300 non-waterproof cellophane was supplied as a substratum for growth, and rolled in such a way that it was both below and above the liquid level.

When isolated in YpSs solution, chytrid growth could be seen on the cellophane with the naked eye after 10 days, but a somewhat longer period elapsed before this was possible when soil extract solution alone was used. The cellophane had become covered with thalli, and these were almost entirely superficial, only the finer terminations of the rhizoids penetrating the substratum. In all, nineteen cultures were selected for detailed examination. Some were perfectly pure, but others were contaminated by bacteria to some extent. However, no account was taken of this contamination if it was at a low level.

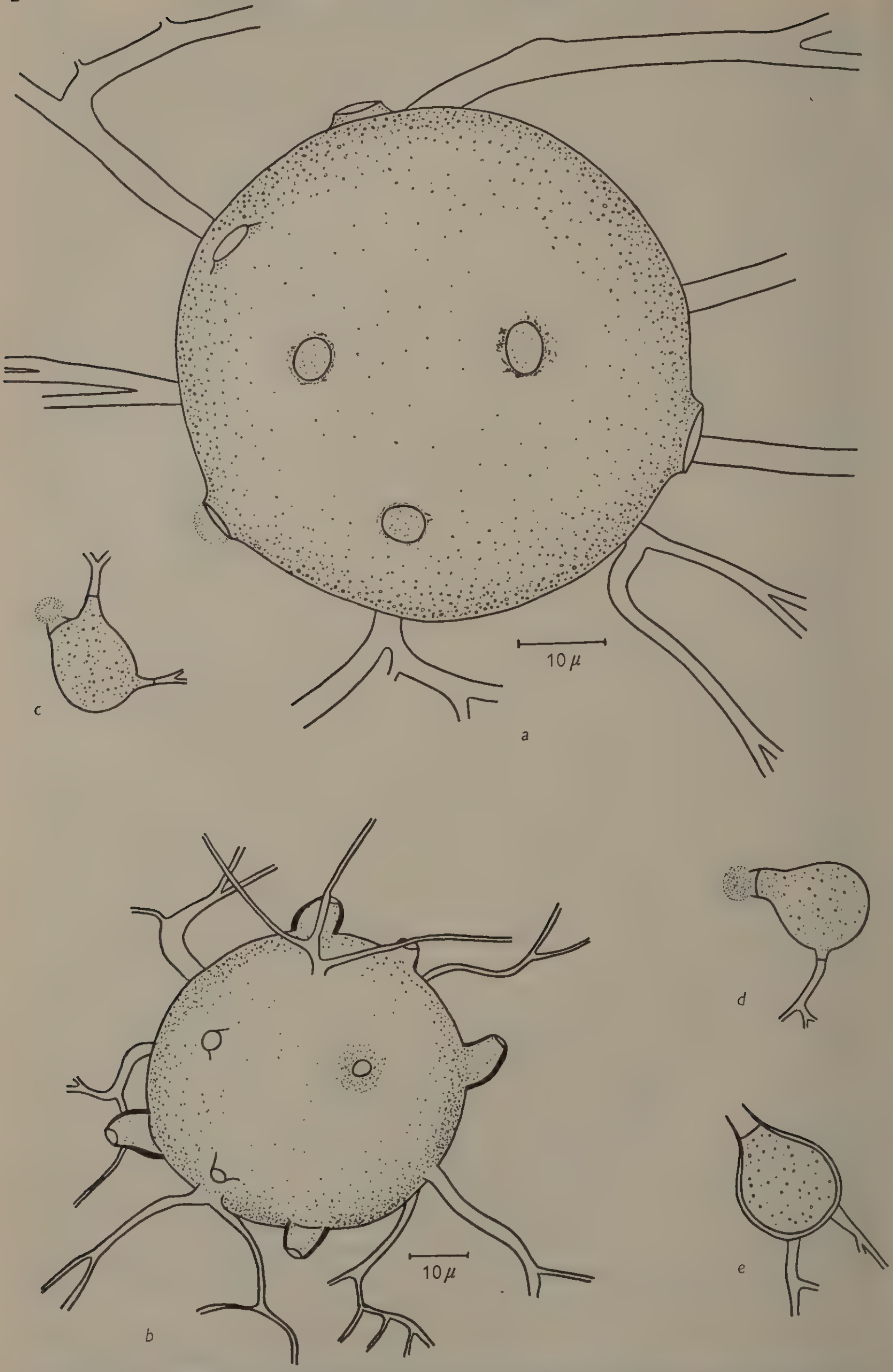
The initial question was whether development was determined by an intrinsic property of the zoospore, or by the type of substratum on which it germinated. Whiffen (1944) has stated that the *Entophlyctis* type of development could have evolved if the inside of the substratum offered a more favourable environment than the outside for the development of the zoosporangium. It therefore appeared to be of interest to determine whether this mechanism was actually operating in *K. rosea*. Each of the nineteen cultures was therefore subcultured on to substrata which it was thought would offer the maximum opportunity for endobiotic development, and all extra nutrients were omitted from the liquid medium supplied. The substrata selected for this purpose were grass leaves and waterproof cellophane. The former encourages the growth of chytrids which penetrate the substratum deeply: the latter was chosen because it was conceivable that the zoospore might germinate on the surface of the waterproofing layer, penetrate it, and form the thallus in the actual cellophane. Also, the endobiotic type of development had occurred in waterproof cellophane in the original soil plates, although there was no penetration of the waterproofing layer under these conditions.

The inoculated cellophane from each McCartney bottle was therefore placed in soil extract solution in a 7 × 5 cm. crystallizing basin with a fitted lid. Bleached grass leaves and P.T. 300 non-waterproof cellophane were added and waterproof cellophane was floated on the surface. The waterproof cellophane was washed on boiling water before use, the other materials were sterilized.

In each of the nineteen isolations, numerous chytrid thalli were found on all the various substrata used. Including the original thallus on agar, at least three distinct generations were represented, and particular attention was directed to young stages to determine the mode of development.

Thalli formed from a swelling on the germ tube produced by the zoospore cyst (the *Entophlyctis* type of development) were never found in these cultures, nor were thalli where only part of the zoospore had expanded. All of the many hundreds of sporangia and resting spores examined had developed by perfectly even enlargement of the zoospore cyst (Pl. 17*b*). It therefore appeared that the type of development exhibited by the thallus was determined by an intrinsic property of the spore rather than by the type of substratum which it encountered. Furthermore, with the material used, and under the conditions stated, the type of development was consistently inherited through a number of generations.

Growth on waterproof cellophane proceeded in exactly the same way as in the initial isolations from soil. The thalli only appeared where the actual cellophane was exposed, and consequently were mostly on the cut

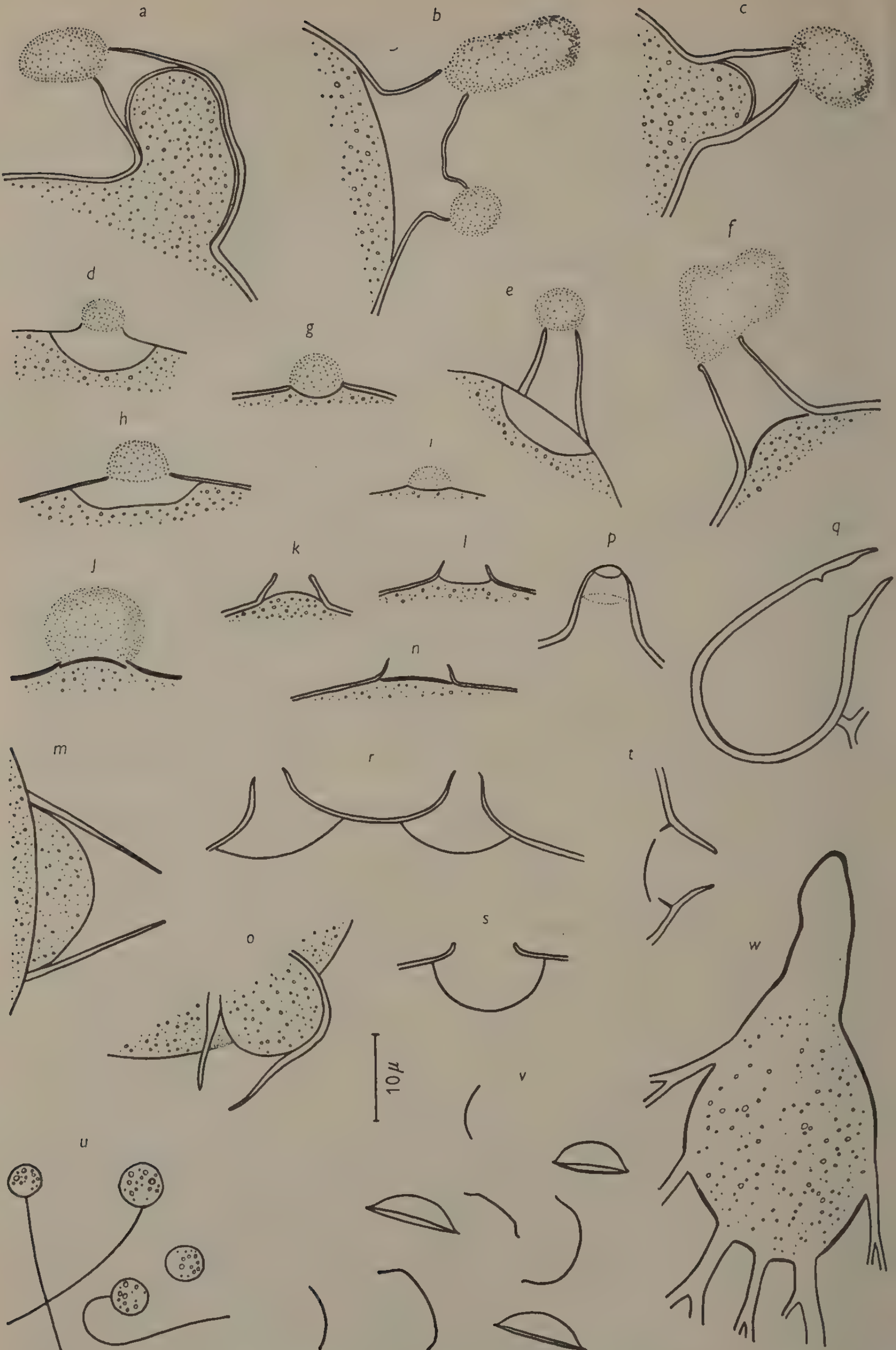


Text-fig. 1. *Karlingia rosea* Johanson. Pure culture material. Typical mature sporangia are illustrated, showing a range of size and form. All drawings at the same scale except *b*.

edges (Pl. 17*e*). Although they were usually completely superficial, a certain number had become embedded in the cellophane to some extent, because their growth resulted in the erosion of the substratum. The thalli were capable of growing on any part of the surface of the grass leaves, and some of the larger sporangia had disrupted the cells of the substratum and become partially immersed in it. Karling (1947) was presumably referring to such thalli when he stated that in *K. rosea* the thallus could sometimes develop partly intramatrically and partly extramatrically. It is felt, however, that in a saprophytic chytrid the precise mode of development from the zoospore is more significant than the final relationship to the substratum.

One possible interpretation of the result I obtained is that in *K. rosea* there are several distinct lines of descent, each different in regard to initial development from the zoospore. I have studied one such line in my cultures, and it is possible that Haskins (1946) studied another. In his unisporangial culture of *Entophlyctis aurea* the sporangia developed from a swelling on the germ tube. Karling (1947) deduced from the description and photographs that this species was endo-operculate and not inoperculate as Haskins believed and was in fact *K. rosea*, quoting his own observations on the latter organism to account for the type of development described. Assuming the species to be *K. rosea*, then Haskins's organism might well represent a line of descent in which 100 % of the thalli develop from a germ tube. It is clear that until more critical work has been done on development in this and other monocentric eucarpic chytrids, it will be difficult to make a full assessment of the value or even usefulness of Whiffen's scheme of classification.

In general there was good agreement between my culture material of *K. rosea* and the material Johanson examined. The main difference was in regard to pigmentation. Johanson's specimens were pink, with the pigment located in the zoospores, but mine were Yellow Ocher (Ridgway) and the pigment was located solely in the walls of the sporangia and rhizoids. Zoospores I observed were 3·7–5 μ in diameter with a number of hyaline refractive granules in the body of the spore and a posterior flagellum 19–21 μ in length (Text-fig. 2*u*). The pigmentation observed applied not only to culture material, but also to the thalli growing on the initial cellophane baits in contact with soil. Thalli grown in the light could not be distinguished on the grounds of pigmentation from those grown in the dark. Sparrow (1948) has described specimens of *K. rosea* from the Marshall Islands as being 'xanthine orange', but it is not clear whether he considered the zoospores to be pigmented or not. Sporangia in my cultures were spherical, 9–140 μ diam., with exit tubes up to twelve in number and 20 μ in length (Text-figs. 1, 2). There appeared to be a correlation between sporangium size and exit tube length, the longer exit tubes occurring on the smaller sporangia and vice versa. Gelatinization of the exit tube apex to form a hyaline plug at an orifice 2·5–6·6 μ diam., and the formation of the endo-operculum below it was a constant feature (Text-fig. 2*a–o*). Occasionally two exit tubes developed in such close proximity that there was only a single endo-operculum for both (Text-fig. 2*b*). Haskins & Weston (1950) have reported that in the '*Rhizophlyctis-Karlingia-Entophlyctis* group' different exit tubes of a single sporangium



Text-fig. 2. *Karlingia rosea* Johanson. *a*, exit tube with wall material deposited round the base in continuity with the endo-operculum. *b–f*, exit tubes with hyaline plugs and endo-opercula. *g–j*, exit pores with hyaline plugs and endo-opercula. *k–o*, exit tubes from the orifice of which the hyaline plug has disappeared, but which are still closed by the endo-operculum. *p*, *q*, exit tubes with the residual rim of the endo-operculum after dehiscence. *r*, *s*, endo-opercula remaining in position after dehiscence. *t*, separation of an endo-operculum. *u*, zoospores. *v*, detached endo-opercula as seen in emptying sporangia, some are drawn in optical section. *w*, young thallus with part of the zoospore cyst unexpanded. All figures except *w* made from pure culture material.

might be exo-operculate, endo-operculate, and inoperculate, but this state of affairs was never observed in my cultures. Exit tubes were often shorter than the minimum figure (7 μ) given by Johanson for her fungus. Occasionally they were completely absent (Text-fig. 2*g*–*j*), in which case the hyaline plug was the only projection from the sporangium wall, and the corresponding endo-operculum was sunk into the body of the sporangium. In the longer exit tubes it was often possible to see that while the convex endo-operculum was being formed there was a simultaneous deposition of wall material round the base of the exit tube in continuity with it (Text-fig. 2*a*).

On dehiscence an initial discharge of zoospores took place through a single exit tube. Zoospores remaining in the sporangium then became motile and the remainder of the exit tubes were utilized for discharge. The endo-opercula were displaced inwards and could sometimes be seen at this stage, carried round by the swirling mass of zoospores (Text-fig. 2*v*). They were of such a delicate nature, however, that they could never be detected in empty sporangia. In Johanson's chytrid a residual rim was left on the exit tube wall after the endo-operculum separated, but this was exceptional in my cultures (Text-fig. 2*p*, *q*, *t*). Usually the outline of the endo-operculum could be followed back to the main sporangium wall, and there was no residual rim after dehiscence.

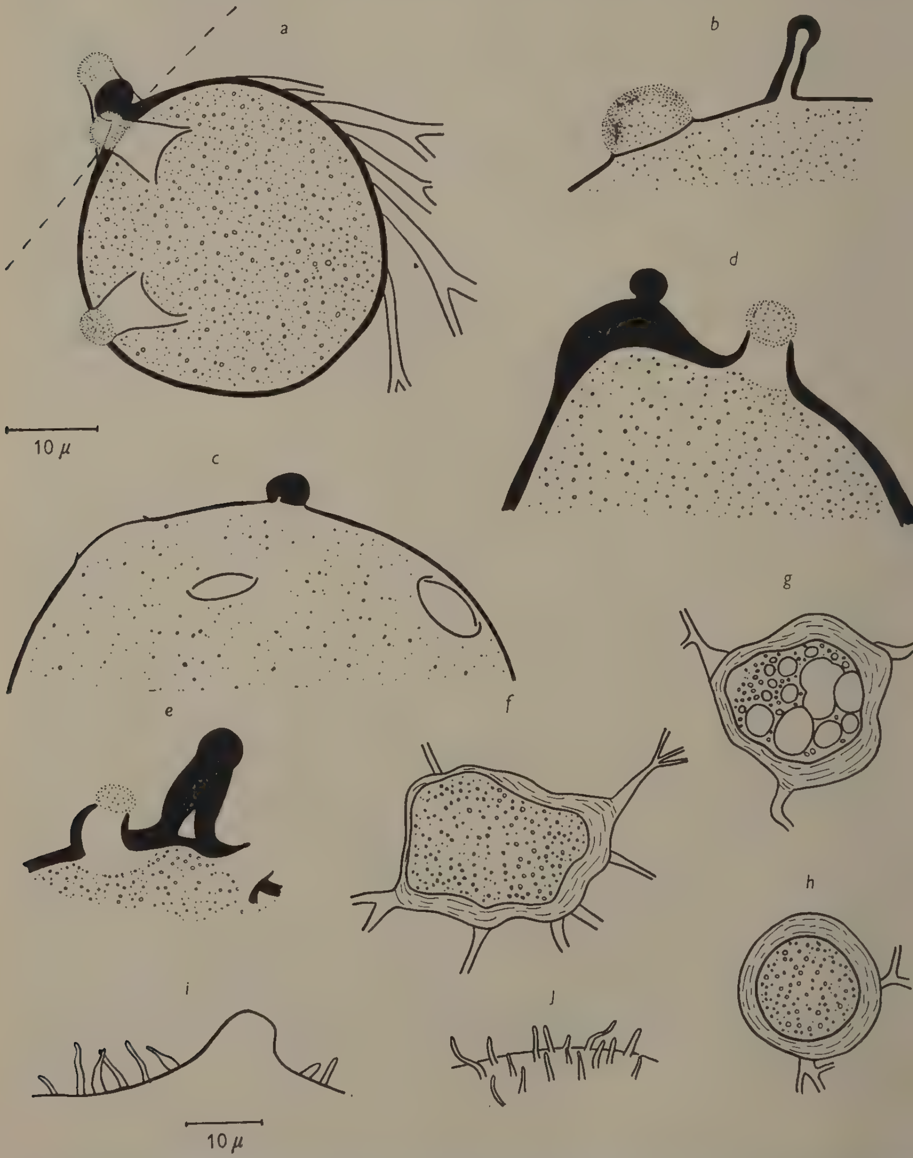
The smaller resting spores in my culture material were spherical (Text-fig. 3*h*), and the larger ones distinctly angular (Text-fig. 3*f*, *g*). They often occurred in clusters as described by Karling (1947). The size range was from 10 μ diam. to 55 × 60 μ, and 17 × 25 μ were the most usual dimensions. They had a thickened wall and contained a number of small oil globules at maturity (Text-fig. 3*f*, *h*).

In addition to the sporangia which have been discussed so far, in which the outer surface was smooth, others obtained in the initial soil plates had a wall ornamentation of unbranched setae which were slightly tapered at the tip and up to 10 μ in length. Again, pigmentation, dehiscence mechanism, and zoospore morphology appeared identical to that described above. In almost all these sporangia a partly or completely unexpanded zoospore cyst was visible (Pl. 17*d*). Karling (1949) has described similar setae in *Truittella setifera*, in which species they developed only in the region of the persistent zoospore cyst and never over the whole surface of the sporangium. In a further thallus type noted (Pl. 17*c*), presumably a resting spore, there was a dense investment of setae but no exit pores. Again the persistent zoospore cyst appeared to be a constant feature.

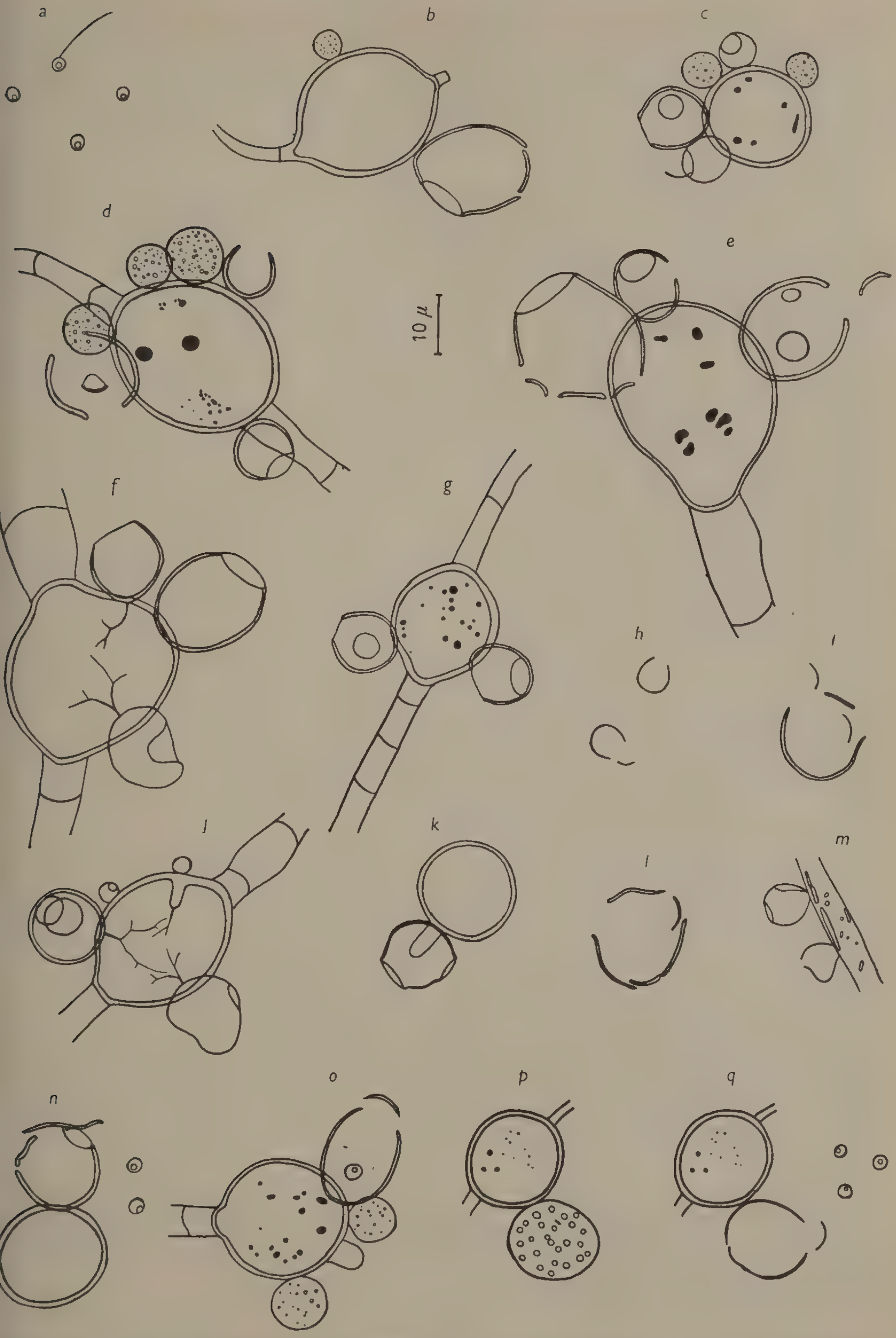
Preliminary investigation at a further site, namely Richmond Park, Surrey, showed that *K. rosea* could again be obtained. Predominant on the initial baits were sporangia of the type described above, with a persistent zoospore cyst and ornamented with setae.

A MULTI-OPERCULATE CHYTRID PARASITIC ON *MUCOR*

When soil from Richmond Park, Surrey, was baited with shrimp chitin (Willoughby, 1957), it was not unusual for Mucoraceous moulds to develop, and they often covered the surface of the plate completely after several days and formed chlamydospores in submerged portions of the mycelium. On one occasion submerged chlamydospores were observed to be heavily parasitized by an operculate monocentric chytrid. The host, *Mucor* sp., was cultured on malt agar, and also grew on shrimp chitin immersed in sterile soil extract solution, indicating its ability to use chitin.



Text-fig. 3. *Karlingia rosea* Johanson. *a–e*, mature sporangia in which a part or the whole of the zoospore cyst remained unexpanded, the cellophane edge is indicated in *a*. *f–h*, restin spores. *i*, *j*, sporangium walls showing ornamentation of setae, the zoospore cyst outline i visible in *i*. *f–h* only are from pure culture material. The scale below *i* applies to *i* and *j* only



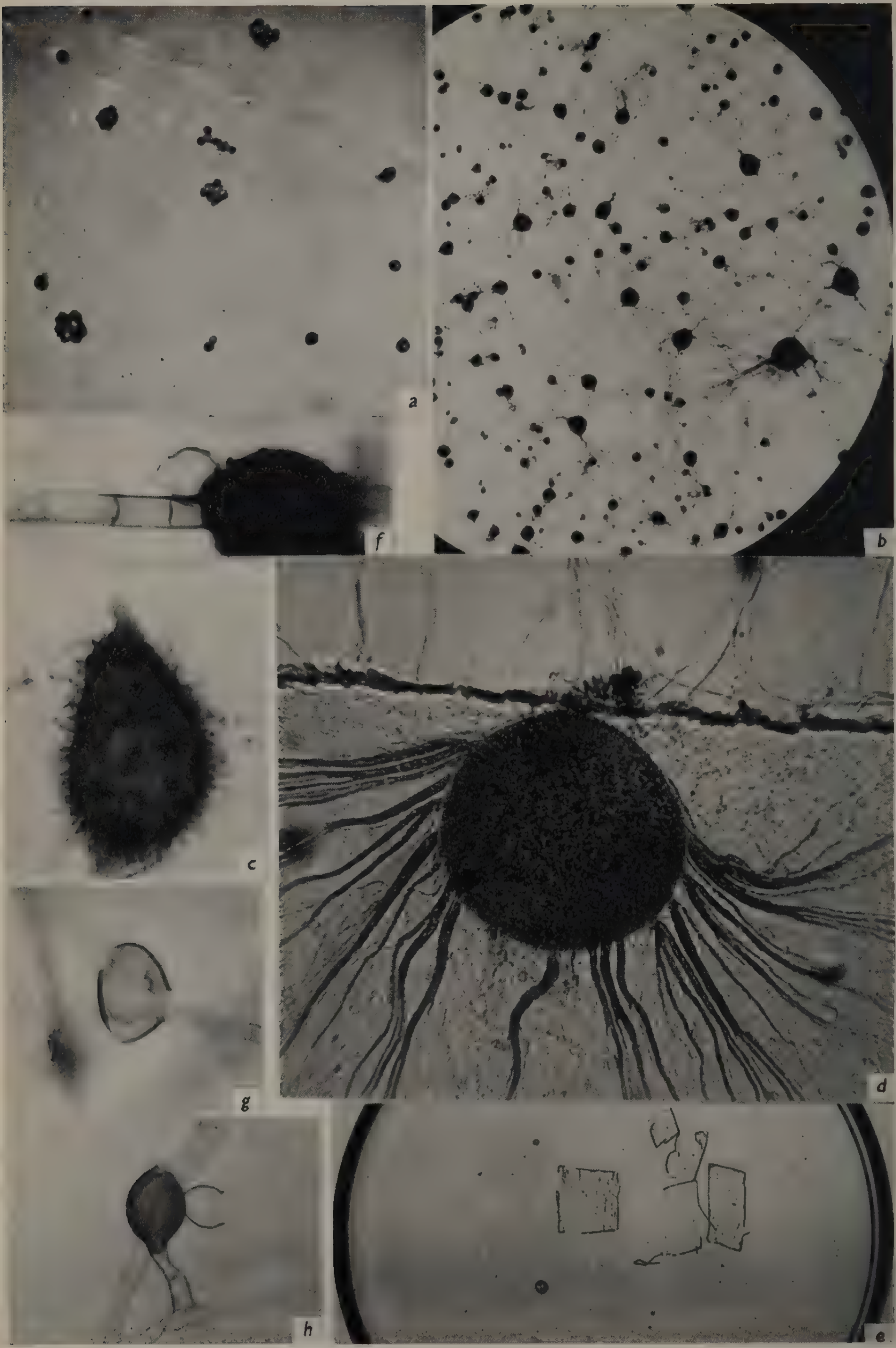
Text-fig. 4. A multi-operculate chytrid parasitic on *Mucor*. *a*, zoospores. *b–g*, *j*, *k*, developmental stages and dehisced sporangia on chlamydospores of the host. Exit orifices of dehisced sporangia are drawn in as plain circles. The sporangium on the left in *j* was observed to shed two opercula at dehiscence. *h*, *i*, *l*, individual dehisced sporangia. *m*, dehisced sporangia on the mycelium of the host. *n*, *o*, dehiscing sporangia. *p*, *q*, the same sporangium before and after dehiscence.

There were often numerous cross walls in the mycelium adjacent to the parasitized chlamydospores (Text-fig. 4*d*, *g*; Pl. 17*f*, *h*), but these were not the result of parasitism as they occurred in the absence of infection. The only previous record of a eucarpic chytrid parasitizing a member of the Mucorales is Karling's (1946) *Rhizophydium mycetophagum*. This species occurred on *Choanephora*.

Zoospores in my chytrid were 2·5 μ diam., with a single oil globule and posterior flagellum 12 μ long (Text-fig. 4*a*). In development the encysted zoospore expanded evenly to give a sporangium, the final shape of which depended on the number of opercula formed. When there was a single operculum it was spherical (Text-fig. 4*d*, *h*) or oval (Text-fig. 4*o*); but the larger sporangia usually had three opercula, and were consequently somewhat irregular in outline (Text-fig. 4*e*, *k*, *l*; Pl. 17*g*). The smallest sporangium observed was 5·5 μ diam., and the largest 23 × 20 μ. Where the rhizoidal system was clearly seen, it was delicately branched inside the host (Text-fig. 4*f*, *j*). When the sporangium had a single operculum, this became completely detached on dehiscence. Most of the zoospores emerged *en masse* with the flagellum coiled round the body of the spore, as has been described in several species of *Chytridium*, and remaining zoospores became motile in the sporangium before escaping. Where there were two opercula either both became detached (Text-fig. 4*j*), or the second was merely displaced. It was not unusual for the displacement of the second operculum to be so slight that the zoospores were unable to escape at this point (Text-fig. 4*n*, *q*). Again, where there were three opercula in the larger sporangia, it was often found that two of them remained more or less in their original positions when dehiscence was over (Text-fig. 4*l*). In some cases it appeared that a certain amount of dissolution had taken place round the rim of the operculum and permitted the zoospores to escape (Text-fig. 4*e*, sporangium on the left).

The question arose as to whether this chytrid is identical with the parasite *Chytridium suburceolatum* previously described from the same site (Willoughby, 1956). The main difference is that in *C. suburceolatum* sporangia with more than one operculum are exceptional, while in the form under discussion sporangia with only a single operculum are in the minority. The fact that two different hosts are involved, however, may account for this difference. On several occasions possible resting spores were found. These were epibiotic, spherical, apparently asexually formed, and 15–22 μ diam. However, since no rhizoidal system could be demonstrated it was felt that further confirmation would be necessary to establish this stage of the life history, and consequently the true generic position.

My thanks are due to Prof. C. T. Ingold for his help and advice with this investigation. The work was carried out with the aid of a maintenance grant from the Department of Scientific and Industrial Research, to whom acknowledgement is made.


a
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EXPLANATION OF PLATE 17

a–e, *Karlingia rosea* Johanson. *f–h*, a multi-operculate chytrid on *Mucor*. *a* and *e* from living material, the others stained with cotton blue. *a*, a zoospore streak made on to YpSs tellurite agar after 6 days growth at 20° C. Individual sporangia such as the ones present in this field were cut out to initiate cultures. In some cases dehiscence has taken place, resulting in the growth of young thalli, clustered round the parent sporangium. (× 75.) *b*, a culture growing on P.T. 300 non-waterproof cellophane. The growth is almost entirely superficial. Many young thalli are present and these have developed by even enlargement of the zoospore cyst. (× 170.) *c*, a thallus with a persistent zoospore cyst and wall ornamentation of setae. No exit tubes are present. It is presumably a resting spore. (× 640.) *d*, a young sporangium growing embedded in cellophane. A persistent zoospore cyst is present, partially obscured at the cellophane edge. Minute setae are also visible in this region. (× 400.) *e*, inoculated waterproof cellophane from a crystallizing basin culture. Four clean pieces of the same material have been added for comparison. Growth is mostly on the edge. In some cases erosion of the substratum has resulted in the formation of floating threads of thalli. (× 0·8.) *f*, *h*, dehisced sporangia attached to chlamydospores of the host. (× 620.) *g*, a dehisced sporangium which became detached from the host in mounting. There were three opercula, two of which are still visible. They were displaced inwards. (× 620.)

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THE ISOLATION AND IDENTIFICATION OF YEASTS CONTAMINATING CATTLE TONGUE EPITHELIAL CULTURES OF THE VIRUS OF FOOT-AND-MOUTH DISEASE

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The methods used in isolating and in identifying yeasts contaminating cultures of the virus of foot-and-mouth disease in strips of epithelium from cattle tongues are described. It is probable that the yeasts studied are representative of the normal flora of the bovine buccal cavity and rumen. Thirteen different species were isolated during a period of 6 months. The most common genera were *Candida* and *Trichosporon*. Three strains of *Pichia farinosa* were identified possessing morphological differences from the type strain of Lodder & Kreger-van Rij.

METHODS

Origin of the yeasts

All the yeasts described in this paper were isolated from virus cultures in which strips or fragments of cattle tongue epithelium were suspended in a medium consisting of Tyrode's solution containing certain amino acids, penicillin and streptomycin. It is assumed that the yeasts were present on the epithelium as all other components of the cultures were sterile. The epithelium was collected in strips with a meat-slicing machine (Frenkel, 1951) from the tongues of cattle within an hour or two of slaughter at a large London abattoir. The strips of tissue were stored until required within the next 1–3 days in Tyrode's solution at 4° C. The breed and age of the cattle from which the tongues were obtained is not known. On a few occasions the tissue was obtained from cattle slaughtered at local abattoirs in Guildford, Aldershot and Reading.

Isolation of the yeasts

The period during which yeasts were isolated was from December 1954 to June 1955. The virus cultures were incubated for 18–24 hr. at 37° C. A sample of the culture liquid was then withdrawn with a sterile Pasteur pipette. Originally, nutrient agar plates and nutrient broth bottles were inoculated but if bacterial contamination of the virus culture was also present the resulting colonies on the nutrient agar plate when first examined at 24 hr. had to be distinguished by microscopical examination. Most of the strains of yeasts were isolated by the better method of using wort agar and wort broth (Oxoid malt extract, Oxo Ltd., London) and incubating at 25° C., conditions under which contaminating bacteria seldom grow.

All strains were subcultured weekly in wort broth and on wort agar at 25° C. and a duplicate culture in wort broth was kept at 4° C. When the identity of a strain was known a streak wort agar slope culture in a screw-capped bottle was stored at 4° C.

Identification of the yeasts

Except for slight modifications, which are mentioned below, identifications were carried out using the methods and media described by Lodder & Kreger-van Rij (1952).

Growth in wort broth. One loopful from a young wort broth culture was inoculated into 10 ml. wort broth. After 2–3 days at 25° C. the culture was examined for presence of ring, pellicle and sediment, and the cells were examined microscopically. The length and width of at least twenty cells were measured. The occurrence of sporing was noted, also the presence of pseudo- or true mycelium. The morphology of the cells was recorded by making camera lucida drawings.

Growth on wort agar. One loopful from a young wort broth culture was streaked on to wort agar in a Petri dish. After 2–3 days at 25° C. the general appearance of the streak was noted. It was examined microscopically for sporing and mycelium.

Growth on potato-agar slides. All strains were examined microscopically after 4–5 days at 25° C. on potato-agar slides. Drawings were made and the presence of true or pseudo-mycelium noted.

Sporulation. Various media were used to induce sporulation. These were gypsum blocks, modified Gorodkowa agar, carrot plugs, sterile water, sodium acetate agar and McKelvey's sporulation medium.

Sodium acetate agar consists of 0·3–0·5 % sodium acetate and 1·5 % agar in distilled water, with the pH adjusted to 6·0. McKelvey's sporulation medium is made as follows: 100 g. scrubbed and grated carrots are pulped and boiled with 1 l. water for 10 min. The pulp is filtered through muslin, and 20 g. agar and 10 g. precipitated calcium sulphate added. This is steamed for 1 hr., dispensed into bottles and autoclaved for 20 min. at 15 lb./sq.in. before sloping.

The shape and size of asci and the number and shape of ascospores were noted. As all plates were incubated upside down, the production of ballistospores was quickly noticed by the formation of mirror images.

Fermentation. 2 % sugar solutions in yeast extract were tested using Durham tubes in screw-capped bottles. The sugars used were glucose, saccharose, galactose, maltose and lactose. If saccharose was fermented 4 % raffinose was also tested. Likewise, if raffinose was fermented 4 % melibiose was tested. As yeast extract by itself was never fermented, it was assumed that none of the strains fermented trehalose, which is present in yeast extract. It was therefore not necessary to use a sugar-free substitute for yeast extract for the basal medium.

Sugar assimilation. Both auxanographic and liquid medium methods were used. In both cases heavy yeast suspensions were prepared as follows: a 3-day-old 10 ml. wort broth culture was centrifuged at about 1500 r.p.m. for 3 min. The supernatant was discarded and the residue resuspended in

5 ml. sterile distilled water. Washing by centrifugation and resuspension in water was twice repeated. Yeast extract was used as a source of vitamins in the auxanographic method. Yeast-nitrogen-base (Difco) was used in the liquid medium test. The sugars used were glucose, saccharose, galactose, maltose, and lactose.

Nitrate assimilation. The methods used were similar to those used in the sugar assimilation tests except that the medium was deficient in nitrogen instead of carbon. The liquid medium test using yeast-carbon-base was tried only if results were doubtful using the auxanographic method. Potassium nitrate was used as the test substance.

Ethanol as sole source of carbon. This test was carried out for all strains but results were of doubtful value.

Splitting of arbutin. This test was used occasionally for further confirmation.

RESULTS

The yeasts isolated and identified are listed in Table 1. The identities of strains A 1, C 2, D 1, J, K, P 1, S 1, 118V and 51 have been confirmed by the curators of the British National collection of Yeast Cultures (The Brewing Industry Research Foundation, Nutfield, Surrey).

Table 1. *Yeasts isolated from contaminated cultures of the virus of foot-and-mouth disease in which cattle tongue epithelium was used*

(The dates are those on which the tongue tissue was collected. Except where otherwise stated this was obtained at a large London abattoir.)

Cryptococcaceae	
Candida tropicalis (Cast.) Berkh.	C 2 (Jan.); F (Feb.); 118 (Mar.), 1955.
C. albicans (Robin) Berkh.	CO 1 (May); H 40 (May); H 10 (May), 1955
C. mycoderma (Reess) Lodder & Kreger-van Rij	L (Feb.); P 2 (Mar.); CO 2 (May), 1955
C. parapsilosis (Ashf.) Langeron & Talice.	NR (Feb.); NS (Feb.); M 5 (May); U 1 (May); U 4 (June), 1955
C. guilliermondii (Cast.) Langeron & Guerra	R 1 (May); S 2 (May); U 3 (June), 1955
C. guilliermondii var. *membranifaciens* (Cast.) Langeron & Guerra	52 (Mar.); T 2 (June), 1955
C. curvata (Diddens & Lodder) Lodder & Kreger-van Rij	P 3 (Mar.), 1955
C. pelliculosa Redaelli	S 1 (May), 1955
Trichosporon cutaneum (de Beurm., Gougerot & Vaucher) Ota	A 1 (Dec.), 1954; JM (local abattoir) (Feb.); P 1 (Mar.); 51 (Mar.); R 4 (May), 1955
T. capitatum Diddens & Lodder	A 3 H (Jan.); D 1 (Jan.); E 1 (Jan.); E 2 (Jan.); O (Feb.), 1955
Rhodotorula mucilaginosa (Jörg.) Harrison	118V (local abattoir) (Mar.), 1955
Endomycetaceae	
Pichia farinosa (Lindner) Hansen	H (local abattoir) (Feb.); CO 3 (May); R 2 (May), 1955
Sporobolomycetaceae	
Sporobolomyces roseus Kluyver & van Niel	Q 1 (Mar.), 1955

Strains C 2, S 1, P 1 and D 1 have been deposited in the National Collection and are catalogued as 470, 471, 472 and 473 respectively. The identities of strains CO 1, M 5, P 3, Q 1, R 1 and T 2 have been confirmed by the Centraalbureau voor Schimmelcultures, Yeast Division, Delft, Holland.

Strains CO 3, H and R 2 possess physiological properties similar to those described for *Pichia farinosa* by Lodder & Kreger-van Rij but they differ morphologically. Strains CO 3 and H are similar and have short-oval to oval cells measuring 2–5 × 3–7·5 μ. Pseudomycelium is formed only sparsely on slide cultures and the asci are usually round to oval. The cells of strain R 2 in wort broth are oval to long-oval, cylindrical or often pear-shaped measuring 2–3·5 × 3·5–12 μ. Strains H and R 2 have been deposited in the Delft collection and are catalogued as CBS 2607 and CBS 2597, respectively.

In addition to the yeasts three isolates were obtained of *Pullularia pullulans*.

DISCUSSION

It is very probable that these yeasts and others constitute the normal yeast flora of the bovine buccal cavity and rumen. It will be seen from Table 1 that non-sporing genera were isolated more frequently than sporing genera. Species of *Candida* and *Trichosporon* appear most frequently among the former. One yeast produced ballistospores, namely *Sporobolomyces roseus*.

The ascosporogenous *Pichia farinosa* was isolated three times. Although the strains differ morphologically from that described by Loder & Kreger-van Rij, there does not appear to be sufficient justification for classifying them as new varieties as their physiological properties are similar.

Di Menna (1955), investigating three different types of soil in New Zealand, found that stockyard soil yielded more yeasts than rural or urban soils. Some of the yeasts isolated from tongue epithelial cultures may well be casual contaminants picked up from the soil in grazing.

A recent study of the yeasts in the bovine caecum (Uden & Sousa, 1957) showed the absence of *Candida albicans* from that habitat. It is interesting to note that this species was isolated three times from tongue epithelial cultures. Vice versa, *Saccharomyces cerevisiae* was isolated nineteen times from the caecum, but not at all from tongue epithelial cultures. However, several yeasts were isolated from both, e.g. *Candida tropicalis*, *C. parapsilosis* and *Trichosporon cutaneum*. Two species of *Trichosporon* (10 strains) were isolated from tongue epithelial cultures compared with one species (5 strains) from the caecum. Two dominant anascosporogenous strains in the caecum *Candida krusei* and *C. utilis* were not isolated from tongue epithelial cultures.

Parle, cited by di Menna (1955), found that *Trichosporon cutaneum* could be isolated from gut contents of sheep and pigs, but *Candida parapsilosis* and *Rhodotorula mucilaginosa* were rarely found in farm stock, although frequently isolated from the air.

It must be mentioned that the genera listed differ from those first reported as having been isolated from contaminated cultures of the virus of foot-and-mouth disease (Wigmore & Henderson, 1955). At that time the identification was at a very incomplete stage.

I wish to acknowledge the help given by Mrs Kreger-van Rij of the Centraalbureau voor Schimmelcultures and by Dr B. L. Brady of the Brewing Industry Research Foundation for advice, for supplying cultures and for confirming results. I am indebted to Mr W. J. Brownsea for technical assistance.

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SPOROPHORE DEVELOPMENT AND PROLIFERATION IN *HYDNUM AURISCALPIUM* FR.

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(With Plates 18 and 19 and 2 Text-figures)

The development and proliferation of sporophores of *Hydnum auriscalpium* on pine cones maintained in damp chambers are described. Investigations indicate that humidity is the most important environmental factor, a R.H. of 95% + being essential for development and proliferation. Extremes of light treatment proved inhibitory to the completion of stipe elongation.

During development there is a change from negative geotropism during stipe development, through the diageotropic reaction of pileus formation to the positive geotropism of the spines. The three responses ensure the vertical alignment which is essential for efficient spore dispersal.

The sporophore is dimitic, the skeletal hyphae of the superficial layer enclosing a central core largely composed of thin-walled generative hyphae. Septate hairs bearing clamp connexions develop from the superficial layer. Proliferation involves the re-organization by the central core of growing apices which then penetrate the superficial layer.

A comparison of the dimensions of the sporophores produced in the field and of those produced in the laboratory suggests that environmental conditions, particularly water supply and humidity, may limit sporophore development in the field.

INTRODUCTION

Several collections of *Hydnum auriscalpium* L. ex Fr. on pine cones were made at Merthyr Mawr, Glamorgan, during November 1956 to March 1957, and the specimens obtained were kept in damp chambers in the laboratory. After intervals varying between 3 weeks and 2 months development of new sporophores and proliferation of mature sporophores were observed. Proliferation was noted by Banker (1906) after specimens had been packed in damp moss in a tin for some time, but on that occasion the proliferations remained epileate. As described by Banker there is, in the Desmazières Pl. Crypt. de France, 954, at least one complete proliferation of a specimen, the new growth arising from the stipe of a sporophore (Pl. 18, fig. 1).

Banker gave only a brief description of his abnormal specimens and that in the Desmazières collection does not appear to have been noted except by Banker, again very briefly. The development and proliferation of sporophores in the present material afforded an opportunity of observing and recording some of the principal stages and more interesting features of those processes.

The influence of certain environmental factors on sporophore development and proliferation, the geotropic reactions during successive phases of development and the relationship between the hyphal systems and sporophore proliferation have been investigated.

METHODS

The majority of the specimens were maintained under very humid conditions in damp chambers, the cones resting in water which was replenished as required, and were subjected to normal diurnal variations in laboratory temperature and light. Selected specimens were exposed to variations in light and humidity in order to determine the influence of these environmental factors on sporophore development. Developmental stages and the results of experimental treatments were recorded photographically.

The proliferations and new growths were examined microscopically, using portions of thick hand sections in which the hyphal systems were teased out. Material from old sporophores was stained using the KOH-Phloxine method, as described by Ragab (1953), and younger material was stained in safranin.

SPOROPHORE DEVELOPMENT

The first appearance of sporophore primordia between the scales of the cones is followed by the elongation of the sporophore initials to a height of 18–42 mm. during 9–35 days. During this phase of growth the initial is white and delicate. The apical growing region persists in this condition, but the more mature parts of the sporophore first become light brown and gradually darken in colour. At the same time hairs develop on the initials, increasing in number and darkening in colour to give the mature sporophore its hirsute character.

The phase of stipe elongation is terminated by the onset of pileus formation the first sign of which is an asymmetric development of the apex of the growing region (Text-fig. 1 *a–c*). The asymmetry becomes increasingly marked, until eventually distinct centrifugal growth commences to one side of the apex. This centrifugal growth takes place in the horizontal plane and results in the formation of a typical reniform or semicircular pileus.

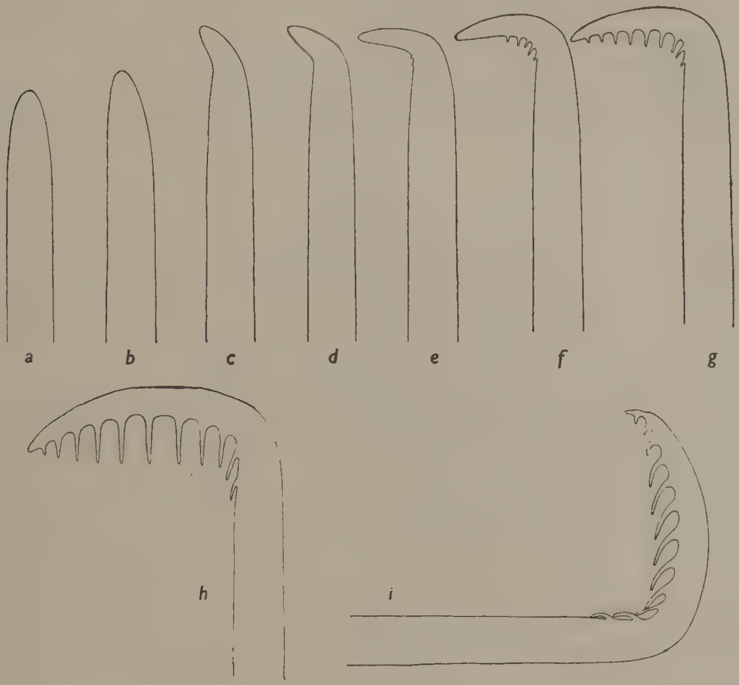
Asymmetrical growth in the vertical plane is usually apparent for less than 48 hr. (frequently about 24 hr.), and following this brief phase centrifugal development lasts 3–6 days. Complete functional maturation of the pileus may take up to 9 days, but basidiospore formation and dispersal commence fairly early (usually within 48–72 hr.) in the centrifugal phase of development.

Formation of the spines over which the hymenium is disposed is largely coincident with the centrifugal phase of pileus formation. While the pileus is still very small and immature, protruberances appear on that region of the stipe adjoining the undersurface of the pileus (Text-fig. 1 *e, f*). As the pileus increases in size so more protuberances appear on its undersurface, spreading from the point of origin of the pileus outwards, and these elongate vertically downwards to form typical spines (Text-fig. 1 *g, h*).

PROLIFERATION

Proliferous growths developed from all parts of the sporophores, including both the upper and lower surfaces of the pileus (Pl. 18, fig. 12). The number of such growths produced on a single sporophore varied between one and more than ten, the majority of specimens bearing four to seven proliferations. Complete proliferations passed through all the phases of normal development described above, but invariably in cases of multiple proliferation only one or two of the numerous growths developed beyond the initial condition, the remainder appearing to be inhibited or starved as a result of the development of the more successful growths (Pl. 18, fig. 2).

A complete secondary proliferation developed in only one specimen. Several proliferations appeared about 2–3 mm. below the pileus on the stipe (Pl. 18, fig. 3) and one of these passed through the phases of normal development to produce a complete sporophore which eventually shed viable spores (Pl. 18, fig. 4).



Text-fig. 1. *a–h*, stages in the development of the pileus and its spines; *i*, spines exhibit positive geotropic curvature when the pileus is placed in the vertical plane.

THE INFLUENCE OF ENVIRONMENTAL FACTORS

Humidity

As described above the new growths and proliferations developed on pine cones standing in water in damp chambers (R.H. 98–100 %). In order to investigate the influence of very high humidity as a formative factor specimens were removed from the damp chambers and maintained outside them at lower humidities (R.H. 65–75 %), but under the same conditions of light and temperature.

The general result of removal from the damp chambers was browning and inactivation of the growing region. In more severe reductions of humidity (to R.H. 50–55 %) the delicate stipe quickly showed signs of collapse. In the majority of cases if such specimens were returned to the damp chamber within 24 hr. then the growing region recovered and elongation continued, but some specimens failed to recover after only 15 hr. exposure to lower humidities.

In two specimens there were interesting sequels to exposure to lower humidities. In both the growing apices became brown in colour and growth ceased temporarily but with their return to the damp chambers both showed renewed activity after periods of quiescence. After several weeks the apex of the growth on the first specimen (Pl. 18, fig. 5) showed a distinct bifurcation whereas the stipe of the second specimen (Pl. 18, fig. 6) produced eight lateral growths after 10 days. In each case the renewed growth was very limited and all remained epileate.

Under laboratory conditions, therefore, high humidity proved essential to the development of the sporophores and temporary reductions of humidity, of short duration, often resulted in inhibition of development.

Light

The influence of light during sporophore development was indicated by the reactions of several specimens to extremes of light treatment.

Continuous darkness proved inhibitory to the development and proliferation of sporophores. The first specimen was enclosed in a tin without a supply of water for 6 weeks. When examined it was found to have at least six proliferations, the two largest appearing on the stipe and the remainder growing from the undersurface of the pileus (Pl. 18, fig. 7).

The growing regions of all the proliferations were light brown in colour, indicating that they were no longer active. The influence of continuous total darkness, coupled with that of the drying out of the cone, had been to inhibit the further development of the proliferations. Following the removal of the cone to a damp chamber, with normal diurnal variations in light, growth recommenced in two of the proliferants and they eventually produced pilei and began to sporulate (Pl. 18, fig. 8).

In a second specimen, the cone bearing two new growths was transferred to a dark chamber and retained there for 10 days during which time the water supply was regularly replenished.

Again it was found that stipe elongation had stopped and that the growing regions had become brown in colour (Pl. 18, fig. 9). In this case, since the water supply and humidity of the chamber were maintained, the inhibition of growth must be attributed to the absence of light. With its return to a normal damp chamber, subject to diurnal variations in light, growth recommenced in one of the stipes and it continued to elongate for several days before becoming finally inactive in the epileate condition (Pl. 18, figs. 10, 11).

The third specimen was subjected in a damp chamber to continuous unilateral illumination from a small light source for a period of 6 days. The growing region showed a very slight positive phototropic reaction but after a few days became brown in colour and ceased to elongate (Pl. 18, fig. 13). Several days after its return to normal diurnal variation of light and darkness the apical region resumed activity and continued to elongate for seven days before becoming inactive once again (Pl. 18, fig. 14).

Thus both continuous illumination and continuous darkness proved inhibitory to the development of new sporophores and proliferations and the growing region showed only a weak positive phototropic reaction to unilateral illumination.

GEOTROPIC REACTIONS DURING DEVELOPMENT

The nature of the geotropic reactions during the successive phases of development was determined by changing the position of the selected specimen in its damp chamber a number of times, each change in position rotating the longitudinal axis of the growing region through 90°.

During the period of elongation the longitudinal axis of the stipe was twice turned through 90°, first about 16 days after the appearance of the primordium and again after a further interval of 9 days. On both occasions re-orientation of the stipe into the vertical position became apparent within 24 hr. indicating a strong negative geotropic reaction during this phase of development. The reactions were confined to the active growing region of the stipe so that after the changes in position had been made the whole length of the stipe indicated the successive reactions to those changes (Pl. 18, fig. 15).

Within 24 hr. of the second change in position formation of the pileus had begun. As in undisturbed specimens the centrifugal growth of the pileus was orientated in the horizontal plane, indicating that the hyphae of the actively growing margins are diageotropic in reaction (Pl. 18, fig. 16).

The developing pileus was retained in this, the normal position for a further 4 days during which time the spines developed as in undisturbed specimens, growing vertically downwards. After 3 days the position of the specimen was changed so as to bring the cap into the vertical plane and the spines into the horizontal plane. Within 24 hr. the spines were again directed vertically downwards and thus more or less lying against the 'undersurface' of the pileus (Pl. 18, fig. 17; Pl. 19, fig. 6). There was no re-orientation of the pileus, which remained in the vertical plane, but after several days the upper edge showed a very limited resumption of growth in the horitzonal plane. Thus geotropic responses were limited in this final phase of development to the spines, which showed a definite positive reaction, and to a remnant zone of activity at the edge of the pileus in which a diageotropic response became evident (Text-fig. 1*i*).

During the development of the sporophore, therefore, there is a transition from a negative geotropic reaction during stipe elongation, through diageotropism during the centrifugal growth phase of pileus formation to a positive reaction during spine formation. Since the last growth phase overlaps the second to a limited extent then two geotropic responses are operative at the same time during the coincident periods of the two phases and the diageotropic response of the extreme edges of the pileus are evident even after spine formation.

Relationship between hyphal systems and proliferation

The hyphal systems of *H. auriscalpium* have been investigated by Ragab (1953) who, using Corner's (1932) terminology, described the species as dimitic, possessing both generative and skeletal systems. These systems he described as 'completely interwoven to form a homogeneous structure throughout the pileus'.

Ragab's study was carried out on dry herbarium specimens of mature sporophores. In the present study microscopic examination of mature and immature sporophores was made using thick hand sections from which portions of the hyphal systems were teased out. Material from old sporophores was stained using the KOH-Phloxine method, as described by Ragab, but younger material was stained in Safranin.

Examination of the hyphal systems showed that in both stipe and pileus they form two well-defined tissues;

(*a*) Superficial layer of unbranched or sparsely branched hyphae, 2·5–3·5 μ diam., having thickened brown walls. From this layer arise the hairs which cover the stipe and upper surface of the pileus. The hairs too are thick-walled, unbranched or sparsely branched septate hyphae with frequent clamp connexions (Pl. 19, fig. 1).

(*b*) Central core of thin-walled, septate, hyaline generative hyphae, 2·5–4 μ diam., with numerous branches, H-connexions and clamp connexions (Pl. 19, fig. 2). In a few very old specimens this central core was found to have degenerated leaving only a small remnant of thick-walled hyphae with clamps. The stipes of such specimens were therefore virtually hollow and readily collapsed.

In the superficial layer the hyphae are aligned longitudinally, i.e. parallel to the surface of the sporophore, but in the central core there is much more lateral development of the hyphal system since the generative hyphae branch more freely. Between the superficial layer and central core lies a 'cortical' zone in which the generative hyphae of the central core give rise to, and are interwoven with, thick-walled, branched hyphae (Pl. 19, fig. 3).

Examination of the spines showed that, in each, thick-walled, slightly flexuous, unbranched and vertically directed skeletal hyphae form the bulk of the growth. Individual generative hyphae grow between them along the length of the spines and eventually turn outwards, horizontally, to produce basidia superficially on the spines (Pl. 19, figs. 4, 5).

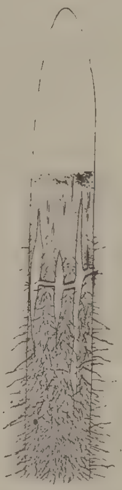
The relationship between sporophore development and proliferation and the hyphal systems has been clearly indicated in the specimens examined. One of the most conspicuous features during the development of both stipe and pileus is the marked transition from the white sappy appearance of the active growing region to the dark brown, hirsute condition of the mature parts of the sporophore. Browning of the superficial layer is associated with thickening of the walls in that layer. Likewise the hairs are white when first produced but darken in colour as their walls thicken.

If normal development is interrupted, as in the specimen kept in continuous darkness, the growing region also becomes brown in colour as thickening of the walls of the superficial hyphae extends to the tip of the growth. In this specimen resumption of active growth followed removal of the inhibitory factor and the superficial hyphae showed obvious signs of disruption, splitting away from the central core as the still viable generative hyphae reorganized an active growing region (Text-fig. 2).

In a second specimen, in which inhibition of growth followed temporary restriction of water supply, the apical growing region again became brown as the walls of the superficial hyphae thickened. With the restoration of water supply growth recommenced, but in the form of lateral branches. These appeared while the hyphae of the superficial layer were still only lightly coloured and there was no obvious disruption of that layer during the emergence of the branches (Pl. 18, fig. 6).

In a third specimen, in which proliferation of the stipe of an old sporophore occurred, numerous primordia appeared in a vertical row down the length of the stipe. The dark brown superficial layer of this specimen was obviously in a more fibrous, rigid condition and the simultaneous production of numerous primordia resulted in the rupture of that layer down the length of the stipe (Pl. 18, fig. 18).

Thus in the development of a sporophore the apical growing region in the active condition is largely composed of thin-walled generative hyphae. As growth proceeds so there is a thickening of walls of the skeletal hyphae, particularly in the superficial layer. Proliferation of the stipe or pileus involves reorganization of new growing regions by the central core of generative tissue and their emergence through the superficial layer may or may not result in its rupture, depending upon the age and degree of thickening of their walls. Finally it seems probable, although very difficult to demonstrate, that under certain conditions individual spines may continue to grow so as to form proliferations from the undersurface of the pileus. This involves a change from positive to negative geotropism as the apical region, after turning through 180°, grows vertically upwards. Proliferations which appear to have originated in this manner have been observed in several specimens (Pl. 18, figs. 7, 12).



Text-fig. 2. Rupture and splitting away of the superficial layer of hyphae due to resumed elongation of the central core (×9).

DISCUSSION

New growths and proliferations were first observed in damp chambers in the laboratory, but a later examination of specimens in the field showed the presence of a number of small proliferations and one large proliferation on sporophores growing from buried cones. Banker's specimens were also found to be proliferous following storage in damp moss in a tin, whereas the Desmazières herbarium specimen would appear to have been collected in the proliferous condition.

Removal of specimens from their damp chambers showed that a very high humidity (R.H. 95–100 %) is an essential pre-requisite for development and proliferation. Where such proliferations occur in nature then it seems probable that surrounding ground vegetation may be instrumental in forming a sheltered, humid micro-climate about the growth. However, even following a very wet spell, the proliferations found in the field were, with one exception, much smaller than those produced in the laboratory.

In complete proliferations the secondary sporophores showed the normal phases of development and normal geotropic reactions during those phases, ranging from the negative geotropism of stipe elongation, through diageotropism during the centrifugal growth phase of pileus development to the positive geotropism of spine formation. In each phase the reaction is confined to the region of active development so that, for example, once pileus formation has begun the stipe loses its power of reorientation. Likewise, following the appearance of spines the pileus loses its ability to return to the horizontal if moved out of that plane. The net result of the three geotropic responses is the vertical alignment of stipe and spines which is essential for efficient spore dispersal.

A number of the sporophores remained epileate, as did Banker's specimens, and the epileate condition had previously been noted by Smith (1908). In the laboratory such epileate specimens were most conspicuous in cases of multiple proliferation of sporophores where usually only one or possibly two of the proliferations appearing on any one sporophore were complete. This would seem to indicate competition between proliferous growths for food and water supplies which must be obtained through the stipe of the original sporophore and which are, therefore, strictly limited in quantity. If the competition for supplies was more evenly balanced in a group of proliferants then all continued to grow for some time but remained epileate, again indicating limitation of food and water supplies. On the other hand, a single proliferation on a sporophore was sufficiently well supplied to produce a complete secondary proliferation on itself.

The limited experimental observations on the influence of light during development indicated that both continuous light and continuous darkness were inhibitory to the completion of stipe development. Obviously the inhibitory effect of continuous darkness cannot be operative during the early stages of stipe formation since sporophores commonly grow from cones buried to a depth of 1 in. in the soil. Also in one specimen a number of proliferations appeared on the sporophore while it was stored in a sealed tin. The inhibitory effect of continuous darkness was demonstrated in a specimen with an elongate stipe; following its removal to a damp chamber growth was resumed.

The hyphal systems comprise a superficial layer of skeletal hyphae on the stipe and upper surface of the pileus, and a central core of generative hyphae with some skeletal hyphae. This differentiation into superficial layer and central core is apparent even in the pileus which Ragab described as 'completely interwoven to form a homogeneous structure throughout the pileus'. The presence of numerous clamp connexions in the thick-walled hyphae of the stipe hairs does not appear to have been noted previously.

A general comparison of old sporophores and those produced in the laboratory, including proliferations, reveals certain interesting features. The old growths and proliferations produced in the field are generally shorter and stouter than those produced in the laboratory and the latter are noticeably more hirsute than the former. This suggests that under natural conditions environmental factors may have a limiting effect upon sporophore development. The present study has not included investigations of temperature effects but a comparison of average maxima and minima in the laboratory and at Glanogwr, the meteorological station nearest to Merthyr Mawr, shows a significant (15–20° F) difference in readings over a period of several months. Of the other environmental factors humidity and water supply would appear to be the most important and probably have the greatest restrictive influence during sporophore development in the field. Adverse environmental conditions may also account for the epileate condition noted by Smith.

It is hoped to carry out more detailed investigations on the influence of environmental factors and the method whereby the growing tips or initials of proliferations are reorganized also remains to be determined.

Thanks are due to Prof. R. Heim, Director of the Cryptogamic Laboratory, Natural History Museum, Paris, for permission to examine and photograph certain of the Desmazières collection.

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EXPLANATION OF PLATES 18 AND 19

Hydnum auriscalpium

(Approximate age of growths indicated in days)

Plate 18

Fig. 1. Herbarium specimen from Desmazières collection showing proliferous growth.
Fig. 2. Single complete proliferation with several inactive initials on the stipe of an old sporophore (20 days).
Fig. 3. Secondary proliferations developing from the stipe of a primary proliferation (7 days).
Fig. 4. Complete secondary proliferation (12 days).
Fig. 5. Bifurcation of a growing apex during stipe development (48 days).
Fig. 6. Development of lateral growths on a stipe (45 days).
Fig. 7. Proliferous growths developing from the stipe and pileus of an old sporophore (25 days).
Fig. 8. Same specimen; two of the proliferations have developed pilei (41 days).
Fig. 9. Two initials, both inhibited by continuous darkness (14 days).
Fig. 10. Elongation resumed in one initial following their return to normal diurnal variation in light (18 days).
Fig. 11. Continued elongation of same initial (26 days).
Fig. 12. Development of proliferation from undersurface of pileus (12 days).
Fig. 13. Growing region showing slight positive phototropic curvature and inactivation following continuous unilateral illumination (22 days).
Fig. 14. Elongation resumed on return to normal diurnal variations in light (32 days).
Fig. 15. Negative geotropic curvature of growth when placed in the horizontal plane (21 days).

Fig. 16. Pileus produced in the horizontal plane following several changes in position during stipe development (26 days).

Fig. 17. Positive geotropic curvature of spines when pileus is placed in the vertical plane (37 days).

Fig. 18. Multiple proliferation of a mature stipe has caused rupture of superficial layer down the length of the stipe (6 days).

Plate 19

Figs. 1–4. Photomicrographs of portions of hyphal systems.
Figs. 5, 6. Photomicrographs of a hand section through a pileus.

Fig. 1. Thick-walled hyphae of the hairs with numerous clamp connexions (× 500).

Fig. 2. Generative hyphae with H-connexions and clamp connexions (× 583).

Fig. 3. Generative hyphae and thick-walled, branched hyphae (× 416).

Fig. 4. Thick-walled, slightly flexuous, unbranched hyphae dissected from a spine. (× 292).

Fig. 5. Single spine showing longitudinal alignment of generative hyphae producing basidia terminally (× 33).

Fig. 6. Section through pileus maintained in the vertical plane showing positive geotropic curvature of the spines (× 13).

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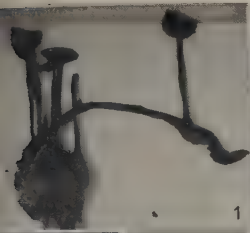
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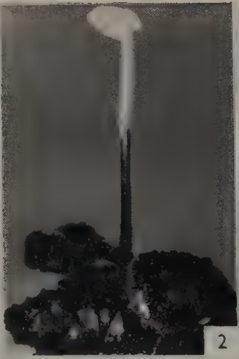
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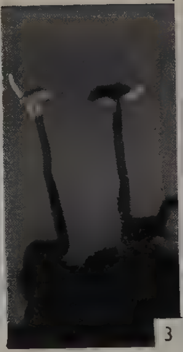
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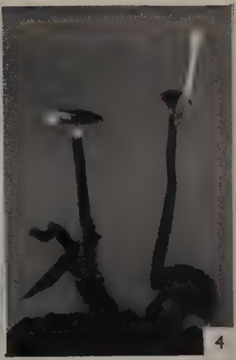
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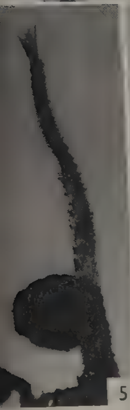
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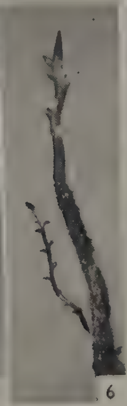
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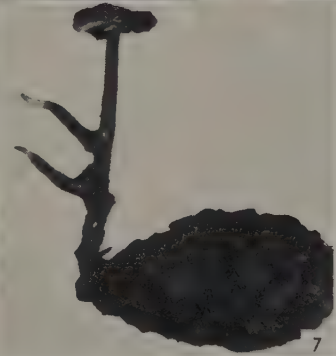
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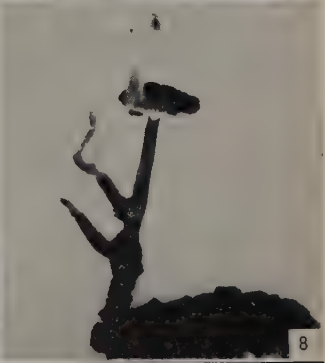
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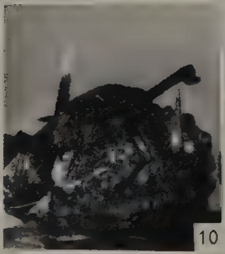
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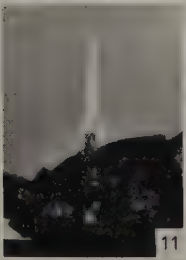
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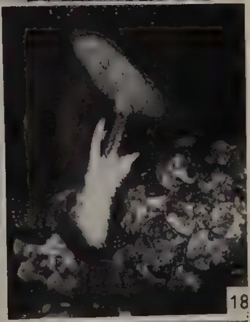
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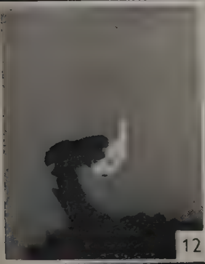
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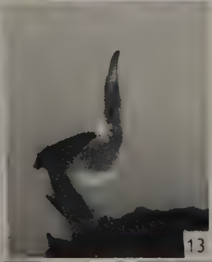
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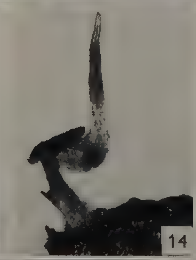
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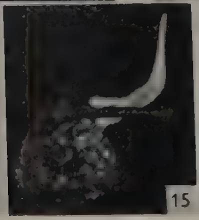
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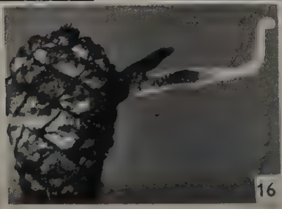
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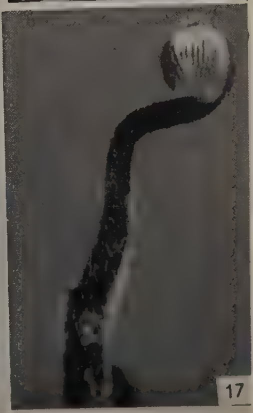
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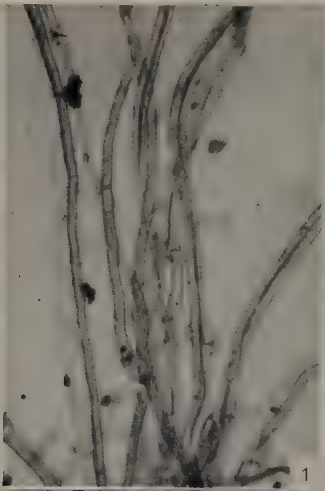
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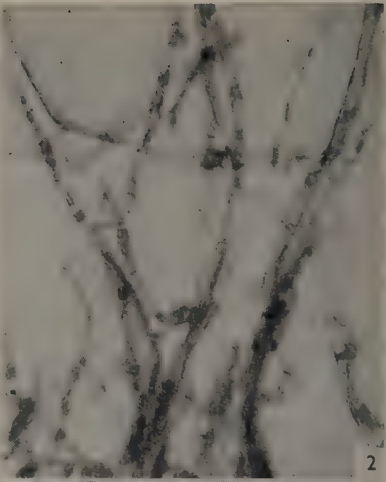
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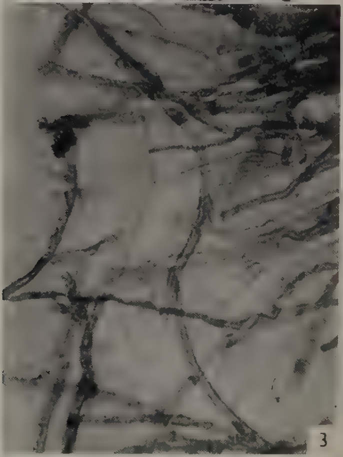
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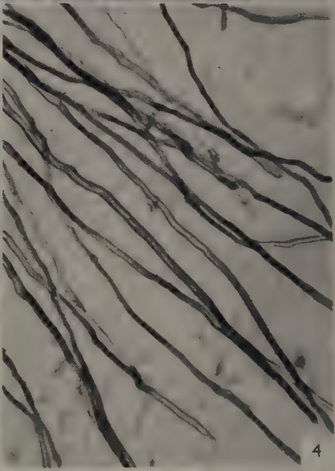
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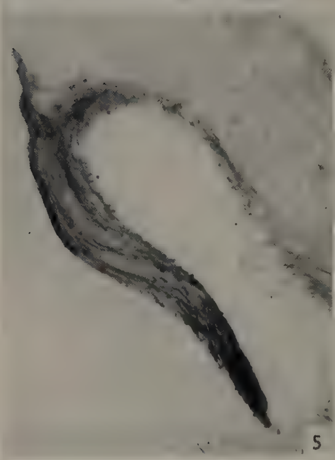
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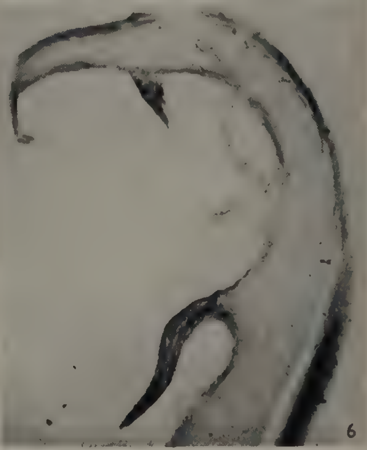
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Trans. Brit. mycol. Soc. **41** (3), 335–340 (1958).

STUDIES OF PYRENOMYCETES

III. *OTTHIA SPIRAEAE* (FUCKEL) FUCKEL, SYN. *DIPLODIA SARMENTORUM* (FR.) FR.

By C. BOOTH

Commonwealth Mycological Institute, Kew

(With 1 Text-figure)

Otthia spiraeae is proposed as the type species of the genus *Otthia*. This species is shown to have pycnidia referred to in recent literature as *Diplodia sarmentorum*.

OTTHIA SPIRAEAE (Fuckel) Fuckel, *Symbolae Mycologicae*, p. 170, 1870 (Fig. 1)

Cucurbitaria spiraeae Fuckel, Fungi Rhenani Exs. No. 975, 1863.
Otthia pruni Fuckel, *Symb. Myc.* p. 169, 1870.
Otthia rosae Fuckel, *Symb. Myc.* p. 169, 1870.
Otthia pyri Fuckel, *Symb. Myc.*, Nachtrg. 1, p. 307, 1871.
Otthia corylina Karst., *Myc. Fenn.*, **2**, p. 59, 1873.
[*Otthia syringae* (Fr.) Niessl, *Hedwigia*, p. 2, 1876.]
Otthia syringae (Fr.) Sacc. *Syll. Fung.* **1**, p. 737, 1882.
Sphaeria sarmentorum Fries, *Syst. Mycol.* **2**, p. 498, 1823.
Sphaeria syringae Fries, *Syst. Mycol.* **2**, p. 492, 1823.
Diplodia sarmentorum (Fr.) Fries, *Summa Veg. Scand.* p. 417, 1846.
Diplodia melaena Lév., *Ann. Sci. Nat.* **5**, p. 292, 1846.
Diplodia rosae West., Herb. Crypt. fasc. 25, No. 1227, 1859.
Diplodia pruni Fuckel, *Symb. Myc.* p. 169, 1870.
Diplodia malorum Fuckel, *Symb. Myc.* p. 395, 1879

PERITHECIA AND PYCNIDIA ON THE HOST

The *ascocarps* develop immediately below the periderm in the cortex of the host. As they enlarge the periderm is raised and finally ruptured, so that the apex of each ascocarp just protrudes. The cortex and underlying wood of the host is heavily invested with dark brown hyphae with cells 5–6 μ in diameter and 18–24 μ long. The ascocarp is stromatic, and typical of the Pseudo-sphaeriales; its wall is 60–76 μ broad and consists of an outer layer of dark brown thick-walled cells 20–22 × 12–16 μ and an inner layer of elongated thin-walled hyaline cells 12–18 × 4–5 μ.

The *asci* which are bitunicate develop from a basal plate and grow upwards between the parallel hyphae; they are cylindrical, contain four to eight obliquely monostichous ascospores and measure 170–220 × 19–26 μ. Spore discharge is usually by the rupture of the outer ascus wall at the apex and the extension of the inner wall, so that it forms a tube stretching towards the ostiole. When the asci are in water the outer wall may rupture in the centre of the ascus, then as the inner wall elongates this upper part of the outer wall remains as a cap on the end of the tube that is formed by the inner wall.

The *ascospores* when young are oval, hyaline and continuous, but become brown and one septate while still in the ascus; they measure 24–32 × 10–14 μ.

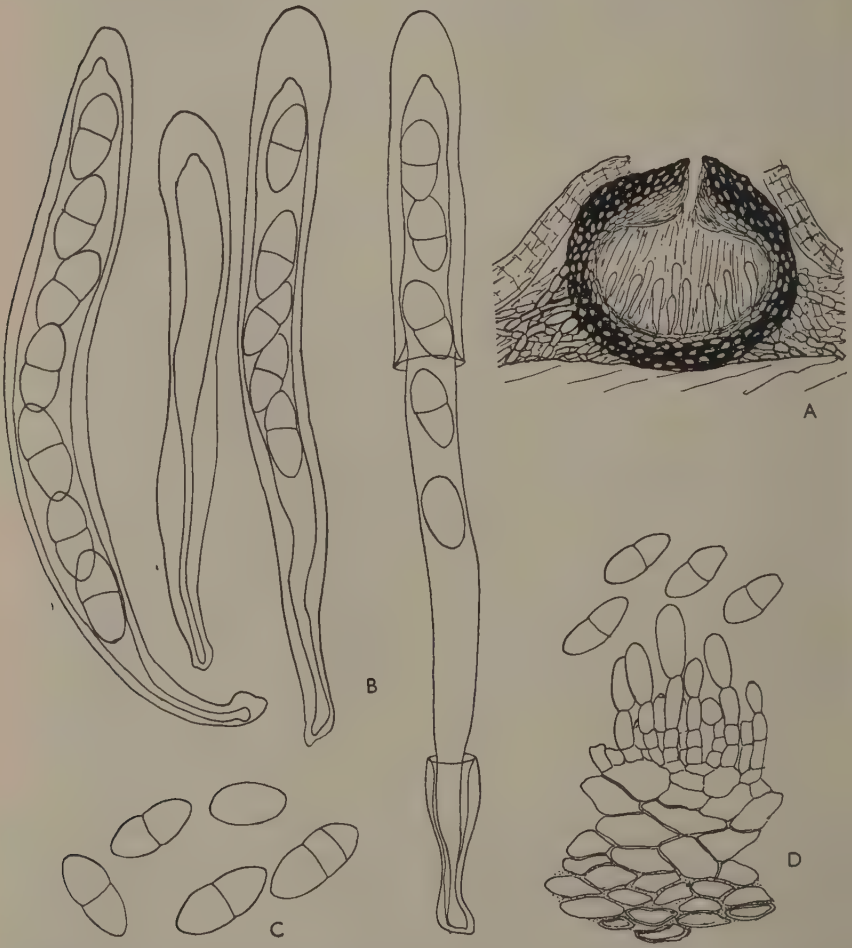

Fig. 1. *Otthia spiraeae.* A, sketch of perithecium in section; B, asci; C, ascospores; D, L.S. through base of pycnidium showing spore formation and liberated pycnospores. A, × 65; B–D, × 500.

CULTURAL CHARACTERS

Cultures were made on 24 August 1956 from a specimen on elm collected by Mr E. A. Ellis in Warwick. The ascospores germinated readily and single ascospore cultures were made, three on corn-meal agar and three on potato-dextrose agar. Six further cultures on corn-meal agar were made on 28 August. Although corn-meal agar will support abundant hyphal growth, typical pycnidia have only developed on autoclaved elm twigs. On corn-meal agar the surface of the culture was covered with a sparse hyaline mycelium 14 days after inoculation; soon small stromatic initials formed and the agar rapidly became darker in colour through the development of dark brown hyphae 4–5 μ wide. These cultures were maintained for 4 months, and by this time they were almost black in colour and with extensive aerial mycelium covering the stromatic pustules. The latter were sectioned and found to have numerous locules in which incipient spore formation had occurred. The cultures made on 28 August and maintained at room temperature in the light showed better stromatic development than the cultures kept in an incubator at 24° C.

Subcultures on sterilized elm twigs in 250 ml. flasks were made from 14-day-old cultures. Growth was rapid, and abundant mature pycnidia were present after 4 weeks. Where these pycnidia had developed on the cut end of the twigs they were superficial but over the parts of the stem covered with periderm they were partially erumpent when mature. They measured 300–420 μ diam. with a wall 20–35 μ thick differentiated into three parts; the outer having thick brown-walled cells 8–12 × 4–5 μ that form directly from the hyphae in the cortex of the host. Where pycnidia develop directly on the surface of the wood, the outer wall is covered with dark brown hyphae with cells 18–24 × 4–5 μ. The central region has thin-walled cells 18–24 × 7–9 μ. In the inner region of the wall the cells are hyaline, globose and 5 μ diam., the surface cells of the inner layer forming the basal cells which give rise to the phialides. The phialides are short, cylindrical, 10–15 × 4–5 μ and finally cover the interior wall of the pycnidium. The conidia are hyaline at first and do not appear to complete their development until they are detached from the phialide. They then become brown and one septate in the locule of the pycnidium before they are extruded; they measure 17–24 × 7–10 μ.

HISTORICAL

The widely distributed pycnidia of *Diplodia sarmentorum* were redescribed by Stevens (1936) when he recorded the species for Britain on *Crataegus*, *Fraxinus* and *Pyrus*, and reviewed its extensive distribution in Europe and its less frequent occurrence in the U.S.A. In Herb. I.M.I. it is represented by fourteen collections on *Acer*, *Cladium*, *Salix*, *Ulmus*, *Citrus*, *Apricot* and *Prunus*; the first four are additional British hosts. It was therefore interesting to find that these pycnidia developed in the single ascospore isolations of *Otthia spiraeae*.

Fuckel erected the genus *Otthia* in 1870 with six species, but did not designate a type. He did refer to respective parts of his Fungi Rhenani as authentic for five of the six species. In four he also cited a *Diplodia* as the pycnidial state of the *Otthia*. However, as in the case of the Tulasnes's researches published several years earlier, the perithecia and pycnidia were linked because of their association on the host and without other evidence or cultural studies. The danger of such assumptions was pointed out by Stevens (1936) when he showed *Physalospora mutila* Stevens to be the ascigerous state of *D. mutila* Fr. Here Stevens stated that the most abundant pycnidia associated with the perithecia of *P. mutila* were those of *D. sarmentorum* Fr. and not, as one would expect, *D. mutila*.

O. syringae was first thought to be based on *Sphaeria syringae* Fries, 1823 Scler. Suec. No. 233, but the three parts of this exsiccatum in Herb. Kew. contain the pycnidia of *O. spiraeae* only. The name *O. syringae* arose when Cooke issued his Fungi Brit. Exs., No. 18, '*Diplodia syringae* Ausw. with *Sphaeria*'. Niessl in 1876 stated that the *Sphaeria* belonged to *Otthia* Fuckel but he made no transfer, nor did he mention the name *O. syringae*. The name was first used by Cooke & Plowright (1879) when they listed it as synonymous with *Cucurbitaria syringae*. The species was first described by Saccardo in 1882 as *O. syringae* (Fr.) Niessl with reference to Niessl's supposed transfer and to Fries's *S. syringae*. Thus Saccardo indicated that *S. syringae* Fr. and *D. syringae* Ausw. were the same species. However, this should be cited as *O. syringae* (Fr.) Sacc. and not Niessl. It is doubtful if Saccardo meant this, for in 1884 he placed *D. syringae* Ausw. as a synonym of *D. lilacis* West. and without reference to *Otthia*.

O. spiraeae is selected as the type species of the genus *Otthia* because it has the oldest epithet amongst Fuckel's original species that can be established without confusion. It was first published with a diagnosis as *Cucurbitaria spiraeae* Fuckel, in 1863.

Fuckel (1870) published the genus *Otthia* with the following diagnosis: 'Perithecia ascophora caespitosa dense gregariaque, carbonacea, papillata, plerumque globosa, vel globoso-conica. Endosporae, uni-raro 2 septatae. Stylosporae didymae, fuscae (Diplodia).'

He described six species in the following order with reference to Fungi Rhenani number where cited:

	Ascosporous	Pycnosporous
Otthia rosae	No. 2025	No. 1564
O. pruni	—	No. 1710
O. populina	No. 966	—
O. spiraeae	No. 975	—
O. urceolata	—	—
O. quercus	No. 534	No. 534

O. rosae and *O. pruni* are identical with *O. spiraeae*.

O. populina (Fr.) Fuckel, although based on *Cucurbitaria populina* Fr. and *S. populina* Pers., is a doubtful species. None of Fries's material has been found and the exsiccatum Fungi Rhenani 966 in Herb. Kew. cited as authentic by Fuckel is not an *Otthia*. The eight specimens labelled *S. populina* in Persoon's herbarium at Leiden include several different species and there is no justification for taking any one of these as the type of the genus. The fifth species, *O. urceolata*, was described without reference to any collection and none has been found. The material in Herb. Kew. labelled 'Herb. Berbey-Boissier ex herb. Fuckel No. 589' contains no *Otthia* ascocarps or pycnidia. *O. quercus*, the material in Herb. Kew., has only the ascocarps of *O. quercus*. The ascospores from these measure 30–36 × 14–16 μ and it is therefore considered to be a distinct species.

O. pyri Fuckel, cited by Clements & Shear (1931), cannot be accepted as the type of the genus as it was not described until 1871. *O. pyri* Fung. Rhen. 2449 is identical with *O. spiraeae*.

H. W. Wollenweber (1941) gave a list of 145 synonyms of *Diplodia sarmentorum* and also suggested but did not prove that *O. spiraeae* was the perithecial state.

SPECIES IN BRITAIN RECORDED AS BELONGING TO THE GENUS *OTTHIA*

Four species of *Otthia* were compiled by Bisby & Mason (1940). The first, *O. syringae*, as stated above is based on a mixed exsiccatum. *Diplodia syringae* Ausw., the species for which the exsiccatum was published, is identical with *O. spiraeae*.

The collection mentioned by Massee (1887) of *O. pruni* on sloe from Eastbourne was found to be identical with *O. spiraeae*, as also was the specimen of *O. crateagi* from Newcastle, Cooke (1890). *O. populina* listed by Massee from Kew Gardens has not been traced. Cooke (1871) had already transferred it to *Cucurbitaria*, but the only British record he cites is Berkeley's in 1838. According to Bisby & Mason (1940) this record of Berkeley's was *Teichospora obducens*.

O. spiraeae is, therefore, the only species of *Otthia* recorded in Britain for which material can be traced.

SPECIMENS EXAMINED

Specimens authentic for name examined in Herb. Kew.

Diplodia malorum Fuckel, Fungi Rhen. No. 1706; *D. melaena* Lév., ex Herb. Berk.; *D. pruni* Fuckel, Fungi Rhen. No. 1710; *D. rosae* Westendorp, Herb. Crypt. Fas. 25, No. 1227; *D. rosarum* Fuckel, Fungi Rhen. No. 1564. *Sphaeria corylina* Karst., F.F. Exs. No. 874; *S. sarmentorum* Fries, Scler. Suec. No. 18; *S. syringae* Fries, Scler. Suec. No. 233. *Cucurbitaria spiraeae* Fuckel, Fungi Rhen. No. 975. *Otthia pyri* Fuckel, Fungi Rhen. No. 2449; *O. rosae* Fuckel, Fungi Rhen. No. 2025.

Otthia spiraeae *folder Herb. I.M.I.*

One collection only is cited for the different hosts. On *Acer*, Selworthy, Somerset, R. McAleer, May 1957 (Herb. I.M.I. 69521); *Cladium*, Wheatfen Broad, Norfolk, E. A. Ellis, May 1947 (15413e); *Prunus*, Cyprus, R. M. Nattrass, July 1931 (6862); *Pyrus*, Malta ex Herb. Caruana-Gatto (70291); *Rosa* ex Herb. Ludwig (6207); *Rubus* Belgium. C. Booth, Sept. 1956 (63776b); *Salix*, Cyprus, R. M. Nattrass, May 1931 (6864); *Ulmus*, Warwick., E. A. Ellis, Aug. 1956 (63581b).

The writer wishes to thank Mr E. W. Mason for his help during the writing of this paper, Dr R. W. G. Dennis for making available the material cited from the Kew Herbarium, and Prof. H. J. Lam of Leyden for forwarding material from Persoon's Herbarium.

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Wollenweber, H. W. (1941). *Diplodia sarmentorum* Fries und ihre Verbreitung. *Z. Bakt.* **103**, 347–357.

(*Accepted for publication* 24 *October* 1957)

Trans. Brit. mycol. Soc. **41** (3), 341–364 (1958).

THE CONSTRICTING RING MECHANISM OF TWO PREDACIOUS HYPHOMYCETES

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(With 6 Text-figures)

The constricting ring mechanism of *Arthrobotrys dactyloides* Drechs. and *Dactylella doedycoides* Drechs., which normally functions in a fraction of a second, has been slowed down approximately 100 times by immersing the rings in concentrated sucrose solution, stimulating them with the same solution at 45° C., and then diluting the medium with distilled water. The phenomenon is described qualitatively and quantitatively with particular reference to changes in the cell wall, osmotic potential and volume of the cells. The previous theories of ring constriction known to the writer and the evidence on which they are based are discussed.

INTRODUCTION

Recent years have seen an increase in the interest taken in the predacious fungi, on which a number of excellent reviews are available (Dollfus, 1946; Drechsler, 1941; Duddington 1955, 1957*a*, *b*). While great impetus to morphological study has been provided by the now classical discoveries of Drechsler in the United States and of Duddington and co-workers in this country, stimulus for parasitological work has been given by Linford in Hawaii and the French School represented by Descazeaux, Deschiens, Roubaud, and others. For details of this work the above-mentioned reviews, particularly that of Duddington (1957*a*), should be consulted.

In contrast, the general physiology of the predacious fungi has been largely neglected. All these fungi possess some specialization which makes it possible for them to catch their prey, kill it and utilize its contents. Probably the most spectacular type of trap is the so-called 'constricting ring' by means of which a number of predacious hyphomycetes catch nematodes. These rings consist generally of three curved cells on a one- or two-celled stalk. They are usually produced at intervals along a fungal hypha and grow at right angles to it. In some fungi these rings are perpendicular to the parent hypha, in others they grow away from it in all directions. If by chance a nematode passes through one, the three cells which make up the ring suddenly inflate so as to obliterate the lumen and thus the nematode is caught, and later penetrated by absorptive hyphae which grow out from the ring cells and appropriate the contents of the animal.

The facts that the whole constriction process takes place in about one-tenth of a second and that during this time the volume of each of the three ring cells more than trebles suggest certain interesting physiological problems. The mechanism has been previously investigated by other workers, but the hypotheses based on their observations have so far not been substantiated.

Couch (1937) observed that rings could be constricted artificially by holding a hot scalpel 2 mm. above the agar surface of the culture for 2 sec., or by applying a drop of warm water (33–75° C.) to the mycelium. Working with *Dactylella bembicodes* he pointed out: 'An examination of the cells which have been caused to swell by dry heat or warm water shows that the three swollen cells contain a refractive substance which is doubtless gelatinous or colloidal in nature. The closure of the trap is due to the sudden swelling of this substance. The heat doubtless "activates" the colloids causing them to swell. This swelling may be due to a *rearrangement* of the molecules of water and colloidal material already in the cell or it might be that additional water is *imbibed from the stalk or thread cells*. The rapidity of the reaction would seem to favour the first view.' Two diagrams are then given, one showing an unconstricted ring without vacuoles and the other a constricted ring exhibiting a large clear area in the centre of each greatly swollen ring cell.

Duddington (1957*a*, p. 69) appears to favour the first view. He remarks: 'It is not suggested that simple intake of water by cell colloids is the motive force behind the swelling, but some kind of rearrangement of the molecules of the colloids probably involving water already present in the cell seems to be the most likely explanation of this remarkable natural phenomenon.'

One year after Couch's observations two French workers, Comandon & de Fonbrune (1938), recorded the constriction of rings of *Dactylaria brochophaga* by cinematography using slow motion.* Following the use of a micromanipulator as stimulating agent they described the process as follows: 'Its (the cell's) volume is more than tripled in one-tenth of a second. Generally a fraction of a second later the other two cells react in the same way and without having been touched. The lumen of the ring is thus completely blocked and the point of the needle is held tightly by the garotte to such an extent that one cannot easily disengage it. The projection of the film which records the phenomenon shows that the volume increase is accompanied by the rapid swelling of several vacuoles in the interior of the ring cells; a swelling which follows, moreover, a certain time (a few seconds) after the start of the explosive dilatation. The interior walls of the three cells appear thinner; if the ring is empty they meet in its centre. These cells which appear to have burst are however not injured. They are the cells which emit the perforating buds and the suctorial hyphae which devour the worm.'

No attempt was made to explain the phenomenon at the time, but in 1939 the same authors wrote: 'We have shown by manipulation with a micro-needle a kinetic response of the protoplasm of the cells of the trap (*D. brochophaga* and *D. bembicodes*) on local irritation of their surface. When the cells are irritated on the inner surface of the ring they swell rapidly and the subsequent volume increase of the vacuoles expresses a sudden

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* This film entitled: 'Recherches experimentales sur les champignons predateurs des nematodes du sol' is available on loan from L'Institut Français du Royaume Uni, 15 Queensberry Place, London, S.W. 7.

modification of either the osmotic pressure of their contents or *of the permeability of their membrane.*'

Two further hypotheses have been suggested privately to the present writer. The first supposes that the increase in cell volume is due to *production of gas* inside the cell, thus avoiding the necessity for absorption of a comparatively large volume of water in a very short time.

The second hypothesis attributes cell inflation to an *intrinsic swelling mechanism in the wall.* This, it is supposed, expands in area and bulges outwards. Thus the protoplast decreases in density instantaneously, under the influence of the reduced pressure so caused within the cell. Pressure equilibrium is later reached by slow diffusion of water into the cell.

Some of these hypotheses are completely devoid of any experimental foundation and all of them introduce a large amount of conjecture. Certain of the observations recorded above will be shown to be erroneous and this will influence the hypotheses based upon them. Thus it was found that the 'refractive substance' referred to by Couch was cell sap. Immediately after stimulation this may contain a large number of protoplasmic granules which show vigorous Brownian movement. Later these granules become deposited on or near the cell wall, leaving a perfectly clear space in the centre of each swollen ring cell. As it then no longer contains any protoplasmic granules, evidence as to its viscosity can not be obtained. Thus Couch on observing the clear central region of the cell interpreted it as a 'doubtless gelatinous or colloidal substance', to which he ascribed the operative function in the inflation process.

Furthermore, certain features of the inflation process were found to be variable; thus vacuole formation in the inflating cells, described by the French workers as *accompanying* the increase in cell volume, is often delayed until several hours after inflation is complete. It cannot therefore be regarded as a fundamental feature of cell inflation. Also the cinematographic technique employed by Comandon & de Fonbrune was not entirely satisfactory, because the film is not sufficiently clear, and with critical illumination more structural detail is visible by microscopical observation. Therefore, because of the variability of the process, it was deemed essential to study a large number of cell inflations, in order to differentiate the fundamental features from occasional or superficial variants. On such a basis of facts it might then be possible to propose a satisfactory hypothesis as to the type of mechanism involved.

MATERIALS AND METHODS

Dactylella doedycoides Drechsler was obtained through the courtesy of Dr J. Juniper (of the Polytechnic, Regent Street, London), who had also identified the fungus. *Arthrobotrys dactyloides* Drechsler was isolated from a plate inoculated with rotten beech wood from the River Mole. The wood containing the fungus was transferred initially to 1 % maize meal agar. It was not possible to prepare unifungal cultures of *A. dactyloides* as the spores would not germinate. If agar blocks of the original plate bearing this fungus were placed on to other preparations bearing as many nematodes as possible, the fungus would spread satisfactorily. It was, however, not possible to obtain sufficient experimental material in this way, and therefore most experiments were conducted with *D. doedycoides*, which agreed well with the original type description of Drechsler (1940). The fungus referred to here as *A. dactyloides* showed certain differences from both Drechsler's description (1937) and Dixon's (1954) only British record. These points of taxonomic interest will be published elsewhere.

Petri dishes containing 15% maize-meal agar were inoculated with *D. doedycoides* and left for *c.* 14 days at laboratory temperature. Constricting rings were then obtained by a modification of Lamy's (1943) method, as follows. Agar strips about 1·5 × 0·3 cm., bearing the fungus, were cut out and placed in 100 ml. 1% horse serum in crystallizing dishes. At this stage no aseptic precautions were taken. The fungus grew out from the agar blocks into the liquid and, after approximately 1 week, a white corona of hyphae was visible to the naked eye. These hyphae bore abundant rings and could easily be stripped off under water and used for experiments.

Preliminary investigations had shown that 1% horse serum, rather than 10% as recommended by Lamy (1943), was the optimum concentration. The horse serum (Evans) was supplied in 10 ml. ampules preserved in 0·15% chlorocresol. In practice, 1 ml. at a time was withdrawn from the ampule, and the latter aseptically resealed.

All drawings were made by means of a camera lucida and all measurements by means of a micrometer slide and eyepiece or by projecting the micrometer slide scale on to the drawing paper. The micromanipulator used was a modification of the one described by Goldacre (1954).

OBSERVATIONS AND RESULTS

Stimulation of the constriction process

After having established a method of growing rings in the necessary quantities a method of stimulation had to be found. In nature, rings constrict when nematodes move into their opening but this method is obviously unsuitable for routine procedure. Couch (1937), working with *D. bembicodes*, had found that dry heat would constrict the rings immediately. Using *D. doedycoides* constriction was similarly produced by holding a hot scalpel near the mycelium.

A drop of water at 50° C. applied to a plate of *A. dactyloides* was found to cause immediate constriction, a result also in agreement with Couch. A gradual heating to 70° C. of the mycelium mounted in water on a slide, under continuous microscopic observation, produced no constriction. The rings of a mycelium heated in this way could not be constricted by a subsequent application of hot water. They had presumably been killed.

The effect of hot water was then more closely studied, using rings of *D. doedycoides*. It appeared that a ring, as soon as it is fully formed, is capable of constriction when stimulated with hot water. If agar blocks bearing fungal mycelium are treated with hot water, the temperature of which is within the range of 31–85° C., constriction is normally produced.

If tufts of mycelium are used the maximum temperature which will induce constriction falls to 54° C., the probable reason being that heat transfer is more rapid and the cells are killed before they have time to constrict. The maximum temperature which still produced constriction appeared to vary with the age of the mycelium, being, in agar block preparations, 85° C. with 4-day-old rings, and 80° C. with 8-day-old rings. If significant, this may indicate a decrease in speed of the reaction, death by heat occurring before constriction has been completed.

Couch did not succeed in inducing constriction by rubbing the inside of the ring with a fine glass hair, whereas Comandon & de Fonbrune (1939) succeeded in doing this with the rings of the same fungus by a very slight stimulation with their very delicate, pneumatic microdissector needle. Their experiment was repeated with both *A. dactyloides* and *D. doedycoides*, using the microdissector referred to in a previous section, and constriction of the rings was achieved in almost all experiments.

The effect of light on rings of *D. doedycoides* was investigated next, because it was thought that very intense light might constrict the rings. A photographic flash bulb was fired 5 cm. from the preparation, and although the experiment was repeated three times on the same sample no constriction was produced. A drop of hot water was subsequently applied and the rings constricted normally. Irradiation with ultra-violet light for 1 min. produced no constriction. The preparation was placed 1 m. away from the source of light, an 'Actina' lamp. Following this treatment, stimulation with hot water (which is normally effective) also failed to induce constriction. The constriction mechanism had apparently been inactivated or desensitized.

Subjecting the mycelium to rapid pressure changes in a Büchner flask by means of a filter pump also failed to cause constriction. It was, however, frequently observed that if a coverslip were dropped rapidly on to tufts of hyphae bearing rings mounted in water, constriction of some rings would follow. It seems therefore that sufficiently high-pressure changes do induce constriction and in the following pages reference is occasionally made to such shock-stimulated rings. It is not always easy to decide whether constriction was due to teasing the material from the agar block preparation in the horse serum, or to the coverslip being dropped on it. It appears that both effects must be considered.

As H. Te Winkel (unpublished) had been successful in inducing constriction by means of an electric induction coil, connected to a pair of microelectrodes, it was proposed to repeat this experiment, but unfortunately no such coil was available. However, the passage of electricity at 12 V. (d.c.) or 240 V. (a.c.), through the water in which the fungus was mounted, failed to induce constriction. The application of a drop of hot water produced constriction following the 12 V. (d.c.) treatment, but after the 240 V. (a.c.) treatment it was ineffective. As in the case of the ultraviolet light, it appears that the application of 240 V. inactivates the constriction mechanism. Application of either voltage directly to a ring by means of two microdissector needles of very thin copper wire similarly failed to induce constriction.

The addition of N hydrochloric acid, N sodium hydroxide, chloroform, ether, dilute and saturated solutions of urea, and benzene to agar block preparations produced no constriction.

So far as these experiments went, therefore, the application of hot water was found to be the simplest means of inducing ring constriction and this method was generally used in studying the cellular changes accompanying the constriction process.

Retardation of the constriction process

As has been pointed out, the constriction process is extremely fast and must be slowed down for study. Comandon & de Fonbrune achieved this by recording the cellular changes on a film using slow motion photography. The disadvantages of this method have been mentioned in the Introduction, and the following alternative method was therefore developed.

A preparation is placed in a watch-glass of freshly prepared 0·3–0·5 M sucrose solution for approximately 1 min. It is then taken out and placed on a slide with a minimum of liquid. Then a drop of the same solution at 45° C. is rapidly placed on the preparation, which is then covered by a circular coverslip. A small drop of distilled water is then placed near the edge of the coverslip and by means of a needle is brought into contact with the edge of the coverslip, under which it will be slowly drawn by surface tension, thus diluting the sucrose solution. Most of the rings will then constrict, the rate of cell inflation being governed by the rate of irrigation. After some practice it is possible to slow down the natural reaction approximately 100 times, i.e. to take place in about 10 sec. This time is sufficient to observe the process in detail and to make rapid sketches by means of a camera lucida.

The drawings thus obtained may be compared with others of constricted and unconstricted rings and 'arrested stages'. These are incomplete constrictions which can be obtained in various ways. If very hot water is placed on the mycelium the ring cells start to swell and are then apparently killed and fixed before the inflation is complete. The rings obtained have a very characteristic appearance in that the three ring cells are all of different size; one cell may be completely inflated and the other two not at all, or one cell may be fully inflated, one slightly swollen and the other half-inflated. In fact all possible variations may occur.

If the fungus is mounted in 50 p.p.m. neutral red for 15 min., and then a drop of water at 45° C. placed on it, the same effect is obtained (fig. 4F). If the fungus is mounted for 15 min. in water saturated with ether, then placed on a slide and stimulated with a drop of water at 45° C., arrested stages are also produced, but all cells in the same ring have reached the same stage and the protoplasts show a plasmolysed appearance (Fig. 4A–C). Arrested stages of the ether type are also found when senile rings are used but in these the protoplast fills the entire cell.

The following sections are mainly concerned with observations on rings treated with sugar solution. The slowed-down constriction seems, as far as can be judged, to be similar to the normal one. The example given in the slowed-down film sequence by Comandon & de Fonbrune is identical with one of the types of constriction described below; the appearance of the fully swollen ring cells is identical with the ones found in nature and there is no reason to assume that the basic mechanism of constriction is different in the presence of sugar solution. The microscopic study as outlined below is thus relevant to the natural process.

Morphological changes in the ring cells

The natural life of a constricting ring may for convenience be separated into a number of stages:

(1) The first is growth. This stage has not been considered here, but it appears that the cells forming the segments of the ring are incapable of inflation until the ring is fully formed. Very occasionally, incompletely developed hooklike formations can be found, and these are not visibly affected by hot water stimulation (Fig. 1 A). The growth stage can be said to be terminated when the ring is morphologically complete and physiologically fully functional.

(2) A fully formed and functional ring may be said to be in the 'pre-inflation' stage, that is the arcuate ring cells are not inflated. As has been pointed out, if a cell is stimulated in suitable sugar solution its volume does not normally alter until the solution is diluted. On stimulation the cell has thus undergone some change, but this may not be detectable and therefore the pre-inflation stage strictly comprises both the unstimulated and the stimulated cell before inflation. Obviously under natural conditions the stimulated pre-inflation stage is extremely short. Its duration under the special experimental conditions devised by the writer has not been recorded but may certainly be in the region of 2–3 min.

(3) The inflation stage covers all stages in volume increase until the maximum volume is reached. Under natural conditions this process is very fast, taking approximately one-tenth of a second, but (as has been described) it can be lengthened to about 10–15 sec.

(4) The post-inflation stage covers all subsequent stages discernible until the fifth stage.

(5) Formation of the infection bulb.

(6) Hyphal penetration. This stage and the preceding one appear to be brought about by direct contact of the inner cell wall with a nematode and have not been observed otherwise. This study is mainly concerned with stages 2–4.

The pre-inflation stage. The rings of *A. dactyloides* and *D. doedycoides* differed lightly both in appearance and dimensions, and it is therefore necessary o describe both types. The rings of *A. dactyloides* grew out from the hyphae as curved hooks, occasionally vacuolated. No granules exhibiting Brownian movement were ever found in these vacuoles and therefore it was not possible to obtain any indication of the viscosity of the fluid. The internal diameter of the ring was 13·5–18·8 μ, the ring cell diameter 5·0–8·0 μ and he ring cell length approximately 22 μ. The rings did not appear to be arranged in any definite order or position but grew out in all directions mmersed in the surface slime of the substrate. Large numbers of nematodes were seen to be caught in these rings by constriction. In the earlier tages of mycelial growth, the worms were trapped around their anterior

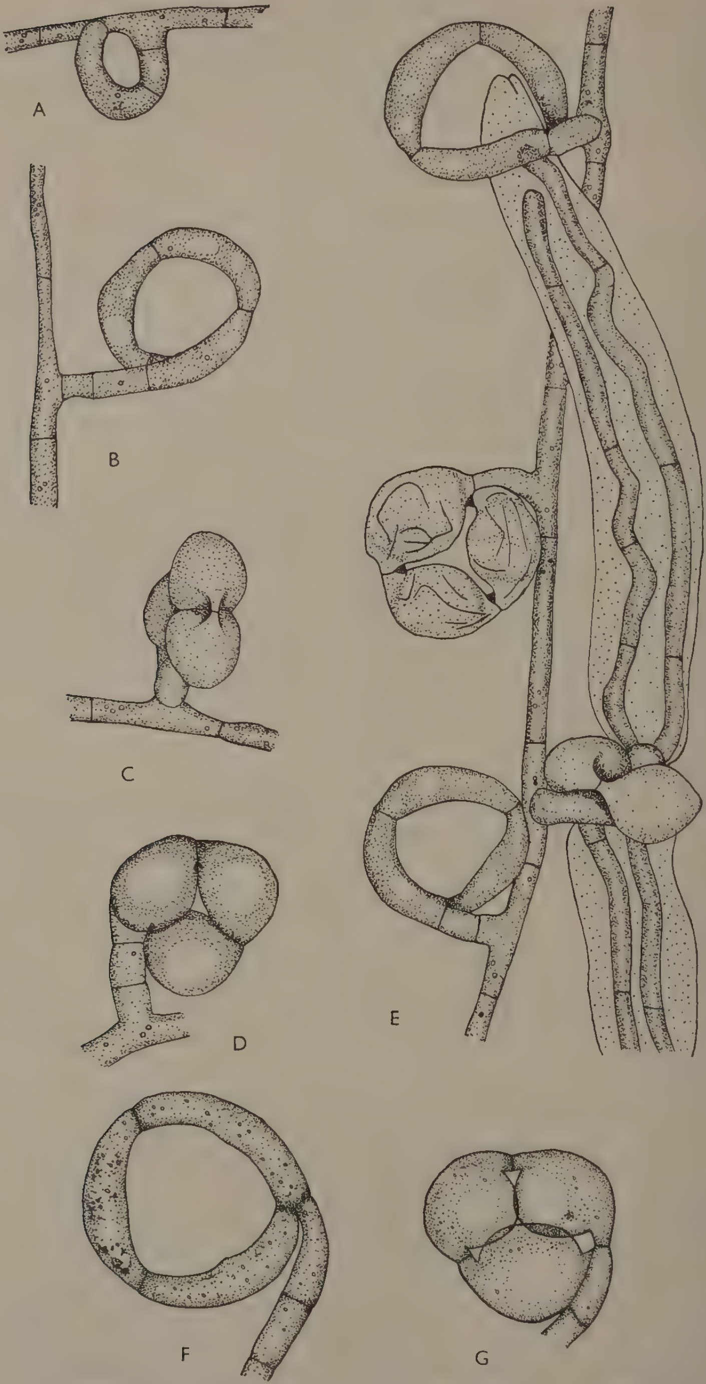


Fig. 1. A–E: *Arthrobotrys dactyloides* Drechs. A, developing ring. B, unconstricted ring. C, D front and side views of constricted rings. E, habit, showing caught nematode pentrated b four absorptive hyphae. Note infection bulb. Two unconstricted and one constricted rin to the left of the fungal hypha. F, G: *Dactylella doedycoides* Drechs. F, unconstricted ring G, constricted ring.

nds, but later around their middles. This would suggest that there is ither an increase in reaction time or a decrease in sensitivity with increasng age of the rings.

Another observation made by the writer in connexion with another redacious fungus, *A. oligospora*, is of relevance here. *A. oligospora* proluces adhesive loops. Very often a nematode was seen to move into a oop and then immediately to retract, moving backwards and curling up, nd then after a second or so resuming forward movement, often in the ame direction as at first, uncurling in the process. If the nematode then ouched the loop again, it would go through the entire avoiding reaction gain until on forward movement it passed the loop without touching it. ither the nematode was aware of the stickiness without actually touching he loops or it was able to detach itself unless it was thoroughly stuck. On ne occasion a nematode was seen to move backwards without coiling up nd it moved into another loop behind it, where it was caught. The ematodes showed the same behaviour in cultures of *A. dactyloides*. There s no evidence, except for an observation by Drechsler on a single ring 1941), that constricting rings are ever adhesive but it does appear that ematodes may somehow sense the danger. On what ring characteristic his perception is based, and how effective it is in nature, is unknown.

As most experiments were conducted with *D. doedycoides*, the rings of this ungus must be somewhat more fully described. *D. doedycoides* did not roduce rings in pure culture but, in the presence of nematodes or on rrigation with dilute horse serum, these were produced abundantly. Jnlike *A. dactyloides*, *D. doedycoides* when grown on a solid medium invaribly produced vertically orientated rings. The rings consisted of 1–3 stalk ells and three ring cells which were hardly ever vacuolated (Fig. 1 F). Very frequently the mature ring cell showed a slight toothlike projection ointing towards the lumen of the ring but this projection was not always learly defined.

The internal diameter of the rings varied from 15 to 20 μ and the naximum diameter of the ring cells from 5 to 9 μ. The ring cells were pproximately 20–30 μ in length.

The most common type of cell exhibited little or no Brownian movement n its protoplasm, which was granular throughout (Figs. 1 F, 2 A). No articles were present which gave a positive iodine or Sudan III reaction. f the preparation was mounted in a 50 p.p.m. aqueous solution of neutral ed chloride, bodies were occasionally found which stained strongly with he dye (Fig. 2 D). These structures occurred almost exclusively in ring ells and were only found once in a normal mycelial cell. They also ccurred in inflated cells where they may appear suspended in the cell and xhibit Brownian movement (Fig. 4 H). It is not known whether they re solid bodies or vesicles filled with liquid in which the dye has preferenially accumulated.

Fig. 2 E shows a ring cell which has been mounted for approximately min. in 0·5 M sucrose and stimulated by a drop of the same solution at 5° C. The granular material was found in the centre of the cell while the est of the protoplasm was almost, but not quite, clear. It is important to ealize that this cell was not plasmolysed. When the cell was slowly

irrigated with distilled water this granular centre produced exceedingly fine vacuoles. This term refers to a clear space which will subsequently be described as a 'vacuole'. At first, it is certainly not bounded by a membrane and thus is not a true vacuole. Whether a membrane is subsequently formed is unknown, but it is certain that protoplasmic granules will, at a later stage, not pass the boundary line between sap and granular protoplasm during the course of their Brownian oscillations, while shortly after inflation there is no such restraint.

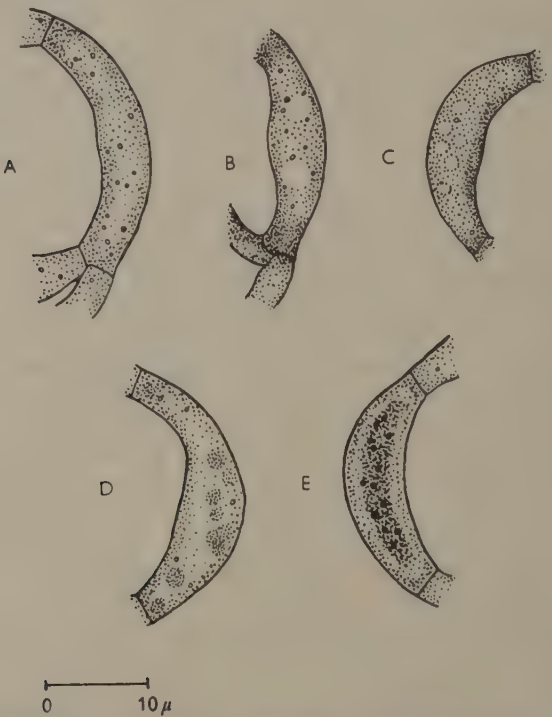


Fig. 2. Pre-inflation stage of ring cells of *D. doedycoides*. A, normal ring cell. B, vacuolated ring cell. C, 'alveolar stage'. D, cell stained with 50 p.p.m. neutral red chloride showing unidentified 'bodies' (see text). E, cell treated with 0·5 M sucrose solution and stimulated with the same solution at 45° C. (see text).

The coarser granules grouped themselves around these swelling 'vacuoles' giving rise to what will be referred to as 'the alveolar stage'. This may be regarded as a stimulated pre-inflation stage (Fig. 2C). If granular and clear zones of protoplasm were separate, as in the instance described above, it could be readily observed that only that part of the protoplasm containing coarse granules would produce 'vacuoles'. The 'alveolar stage' was quite readily obtainable when cell inflation was retarded by means of sugar solution. It seemed a rather stable stage; the 'vacuolar' size was similar in all cells and coalescing of 'vacuoles' was not observed. The wall was not blown out (Fig. 2C). Ring cells of *D. doedycoides* which contained large 'vacuoles' were very rare indeed (Fig. 2B).

The inflation stage. (*a*) *Protoplast and cell changes.* Series I (Fig. 3) was obtained by mounting the fungus in 0·5 M sucrose solution for 1 min. followed by stimulation with the same liquid at 45° C. and irrigation subsequently with distilled water. The small, clear, vacuole-like regions in the protoplasm of the 'alveolar' stage increased in size and the inner cell wall bulged out. As the cell size increased, some 'vacuoles' coalesced and Brownian movement in the whole protoplasm started. This became more and more vigorous. When full inflation was reached, this movement of the protoplasmic granules was exceedingly vigorous and four large 'vacuoles' occupied a considerable portion of the cell volume.

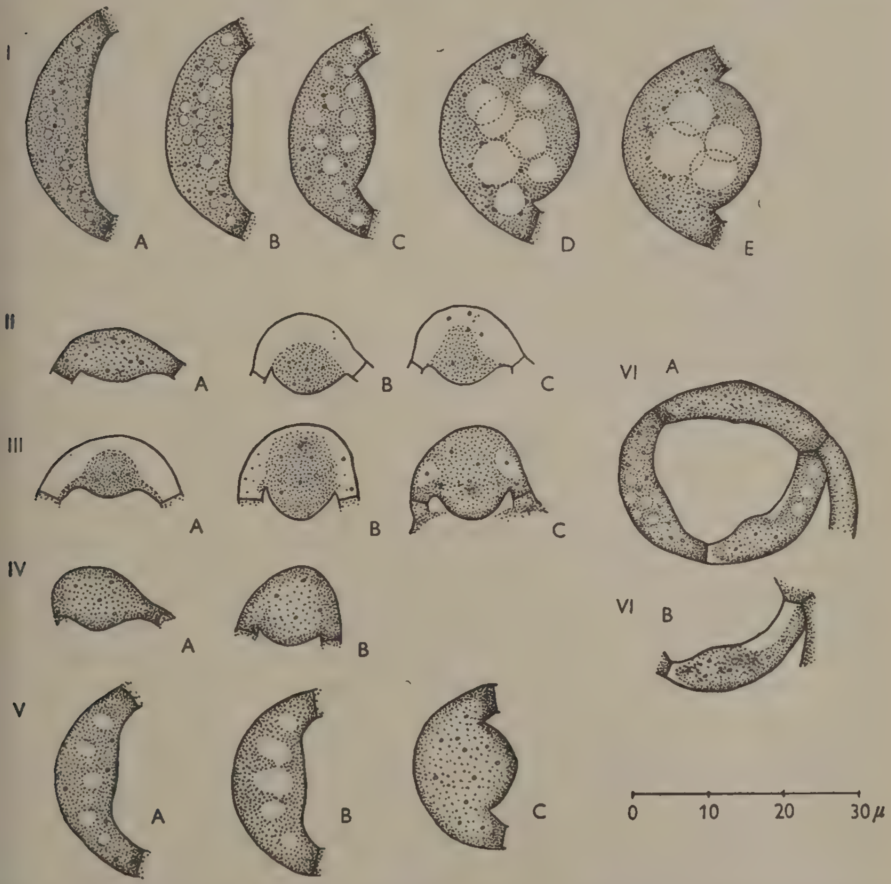


Fig. 3. Inflation of ring cells of *D. doedycoides*. Series I, 'vacuolar' accumulation of liquid. Series II and III, accumulation of liquid outside the granular protoplasm. Series IV, even protoplasmic dilution without formation of clear areas ('vacuoles'). Series V, essentially a combination of Series I and IV. Series VI, A, ring showing 'alveolar', unstimulated and slightly inflated cells. Series VI, B shows transfer of liquid from 'vacuoles' to cell periphery (see text).

Series II in Fig. 3 (stimulation at 48° C., 0·3 M sucrose) shows the same process except for the fact that no 'vacuoles' were formed and the water accumulated outside the mass of protoplasmic granules but presumably within the plasma membrane.

Fig. II A in Fig. 3 shows a slightly swollen ring cell containing granular protoplasm in which Brownian movement did not appear. On irrigation the cell increased in volume, the inner wall bulging out more and the area of the other walls increasing simultaneously. The protoplasm was attached to the inner wall and no Brownian movement occurred (II B). The whole process lasted approximately 5 sec. During the next 3–5 min., vigorous Brownian movement occurred at the outer protoplasmic limits and granules detached themselves. The granular region of the protoplasm appeared to 'invade' the liquid space around it, but most of the granules remained apparently attached to each other by a low viscosity matrix. The further apart the granules appeared to be, the more vigorous was their Brownian movement. A few granules had become completely detached and oscillated freely in the clear liquid, which implied the absence of a membrane between the two regions (II C). The Brownian movement of the protoplasmic granules in this type of cell inflation is thus an indication of the rate of intermixing of the two liquids.

It is important to appreciate that the condition illustrated in Series II is not to be interpreted as one of plasmolysis. If an isolated cell is plasmolysed, the protoplast shrinks away from the cell wall and possesses a definite boundary. No granules ever separate from it and move into the liquid space between the wall and the protoplasmic membrane. If a cell inflates, the membrane appears to adhere to the cell wall and within it the protoplasm exhibits clear water-containing and granular regions. The water may be contained in circular pockets or 'vacuoles' (Series I and V) or may appear to dilute the protoplasmic sap without forming definite 'vacuoles' as in the example just given. In almost all instances, however, the water aggregates ultimately in the centre of the cell, giving rise to a single large 'vacuole'. As this 'vacuole' formation occurs after full size is reached it will be described under the heading of post-inflation changes in the following section.

Series III (Fig. 3) shows essentially the same process. The protoplast exhibited very little Brownian movement in the initial stage (III A), but this became increasingly vigorous as inflation proceeded. Stage III C followed III B after 10 min. In III C Brownian movement was exceedingly vigorous, but all granules, except one which was oscillating freely in the left-hand 'vacuole', formed a coherent mass of low viscosity. Series IV (Fig. 3) shows a common type of the same process. There was little Brownian movement in the slightly inflated stage and very vigorous Brownian movement in the fully inflated cell. No 'vacuoles' were present.

Series V (Fig. 3) shows in V A a slightly swollen 'vacuolated' cell. In one of the 'vacuoles' a protoplasmic granule, which had become detached, showed vigorous Brownian movement in contrast to the others in the granular region of the protoplasm, which showed none. On inflation, the 'vacuoles' increased in size and the protoplasm between them was stretched. These strands at first appeared 'stringy' like white of egg, as in V B, but as inflation proceeded further they first tore apart and then broke down altogether, the 'vacuoles' disappeared and the whole cell became filled with vigorously oscillating granules. The final appearance was identical with IVB.

Series VI (Fig. 3) shows a ring cell in a preparation which had been mounted in water and stimulated probably by shock. The initial appearance of the whole ring is shown in VIA. One ring cell has a normal, non-stimulated appearance, one, although not inflated, is of the 'alveolar' type, while the third shows incipient inflation. When the 'vacuoles' in this cell disappeared (within about 1 min.) the granular region of the protoplast shrank also but the cell dimensions were unchanged. This particular type of behaviour is, without much doubt, a freak. None the less it shows quite nicely that under certain circumstances the liquid contained in the protoplasm can pass from central 'vacuoles' to the periphery, so adding to this outer clear region between the granular cytoplasm and the cell wall. This transfer may occur without change in the total volume of the cell.

Fig. 4A–C shows stages which have been arrested by treatment with ether dissolved in water before stimulation with hot water. Noteworthy is the relatively small size of the granular region of the protoplast as compared with the actual size of the whole ring cell. The significance of this and of the position of the plasma membrane is not known; presumably the outer boundary of the protoplast is still adjacent to the cell wall. Ether is known to increase the permeability of cell membranes. It is possible that the rings constricted normally after the initial stimulus but then collapsed due to the outward leakage of water. In any event the phenomenon took place between stimulation and subsequent microscopical observation and it was not possible to observe it.

(*b*) *Wall changes* It was thought that as the ring cell becomes inflated the microfibrillar arrangement of its wall might change. A preparation of *A. dactyloides* was therefore observed under the polarizing microscope. The ring cells were birefringent and constricted during observation, presumably due to the local heating, but no change could be observed in the wall. This observation could not be repeated as a polarizing microscope was no longer available. The observation may not be definitely conclusive as a certain amount of extraneous birefringent material was present on the slide, so that small changes in the optical properties of the ring cell wall might well pass unnoticed.

Observations, both by Comandon & de Fonbrune (1938) and by the present writer, on the wall changes in *D. doedycoides* have shown conclusively that the inner ring cell wall decreases in thickness during inflation. This is to be expected as the area of the wall increases considerably at this point. In some cells an additional thickening of the inner cell wall was shown at the junction of the cells (Fig. 4D; arrested stage, hot water at 48° C., in presence of ether). In other cells an inner annular thickening can be seen at each end of the inflated cells (Fig. 4E). This is detectable in the film of Comandon & de Fonbrune, although they make no reference to it. This annular thickening was almost universally observed in some trials and completely absent in others. No rule for this was found but the thickening appeared to be absent when a ring cell was inflated slowly. It occurred when cells swelled and then were arrested. It was not seen in completely inflated cells. Possibly some slipping of cell-wall microfibrils may occur during cell inflation. Such a fibrillar aggregate may at first be visible as a ring and later disappear on further inflation. A further discontinuity in wall properties, giving rise to sharp re-entrant angles of the inflated cell near the septa, is apparent from Figs. 1G, 4N and 4K. In fact the area of the wall which does change markedly is limited and is shown diagrammatically in Fig. 6C, D. The other parts of the wall appear rigid, and on inflation these parts are apparently strained. The behaviour of this part of the wall may be compared with a bow when its bow-string is stretched. This simile should, however, not suggest that this part of the wall is entirely passive. It may well contribute wall material to the actively stretching part in submicroscopic aggregates.

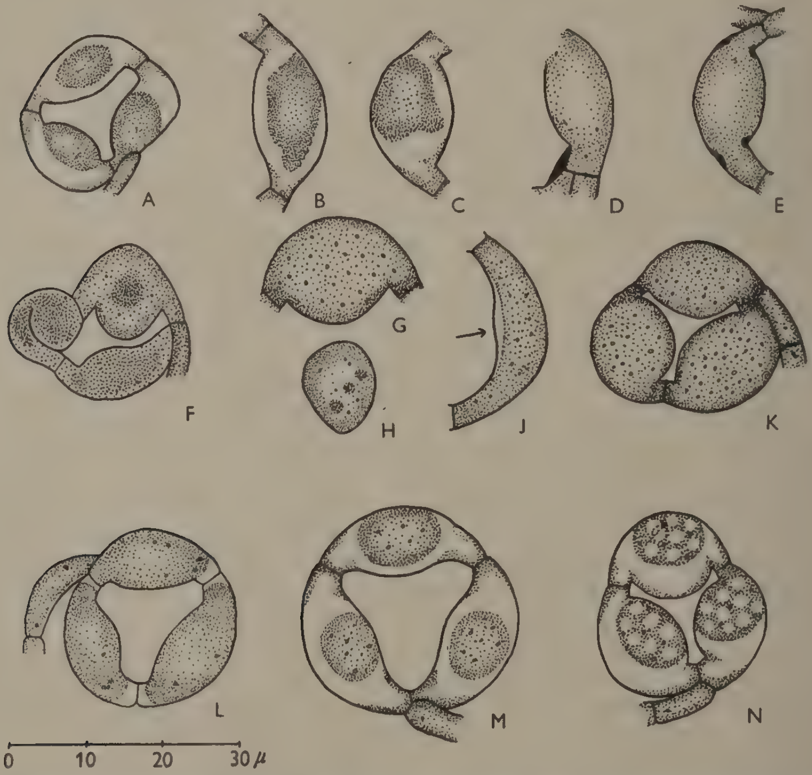


Fig. 4. *Dactylella doedycoides*. A, B, C, complete ring and single ring cells treated with ether before stimulation. D, ring cell showing thickened wall portion. E, ring cell showing annular thickening of cell wall. F, incomplete constriction obtained by mounting rings for 15 min. in 50 p.p.m. neutral red chloride followed by heat stimulation (see text). G, fully inflated ring cell. H, fully inflated ring cell showing the same unidentified bodies depicted in fig. 2D (50 p.p.m. neutral red). J, uninflated cell showing incipient plasmolysis indicated by arrow. K, constricted ring immersed in 0·5 M sucrose showing slight opening. L, M, N, plasmolysed constricted rings (see text).

Under natural conditions inflation is irreversible. If, however, an inflated cell is plasmolysed the ring will open a little as the cell walls collapse slightly or are drawn inwards by adherence to the protoplast. The extent of this phenomenon is variable. In some instances the ring will open ust a little and the protoplast contracts immediately (Fig. 4N; this ring was constricted with water at 45° C., and then observed in 0·7 M sucrose). In others the protoplast will draw the adhering wall with it for quite a considerable distance before the wall retains its shape and the protoplast contracts away from it (Fig. 4L). Although the wall changes are permanent, and due to plastic flow, some elasticity is therefore still discernible. It appears that the longer a ring cell remains in an inflated condition, the greater is the loss of elasticity of its wall.

The fact that after inflation the cell content is capable of plasmolysis points to an extraordinary degree of plasma-membrane elasticity. If protoplasts of onion epidermis are floated out of their cell walls into suitable solution they will burst if a volume increase of about 25 % takes place (Levitt, Scarth & Gibbs, 1936). On inflation the cell volume more than trebles as will be shown below, but none the less the membrane either survives this stretching quite intact or it is immediately repaired.

The post-inflation stage. Fig. 4G represents a typical cell at the end of the inflation. Brownian movement is exceedingly vigorous and no 'vacuoles' are present. Occasionally the same large solid bodies as described in the pre-inflation stage (Fig. 2D) can be detected by staining with neutral red (Fig. 4H) and they oscillate slightly within the cell. Cells have been observed to remain in this condition for up to 1 hr. following stimulation by a drop of hot water. Usually, however, further changes occur, giving rise to certain characteristic post-inflation stages. These were studied by microscopical observation of heat-inflated cells for up to 1 hr. or more after inflation. The exact course of the changes may be variable, but generally one single 'vacuole' is produced and the Brownian movement of the protoplasmic granules ceases. This cessation of Brownian movement will be referred to as 'protoplasmic setting'. Series I, II, III and V of Fig. 5 show the usual course of 'vacuole' formation, which takes from 1 to 15 min.

In Fig. 5, Series I, the granules 'set' along the walls of the cells and a clear space, the 'vacuole', slowly delimits in the centre of the cell. Occasionally a number of 'vacuoles' are formed initially but these rapidly coalesce (Fig. 5, Series II and V). During the early stages of 'vacuole' formation, the interphase between cytoplasm and 'vacuole' is practically invisible. Later it appears clearly. The 'vacuole' may shift during its formation to the excentric or endocentric position. It appears that (in nature) when a nematode has been caught, the 'vacuole' invariably ends up excentrically (Fig. 5, Series III).

Cessation of Brownian movement appears always to originate on the opposite side of the 'vacuole' and near the cell wall (Fig. 5, Series III). Any granules left in the 'vacuole' (this is exceedingly rare) exhibit vigorous Brownian movement. Occasionally the 'vacuolar' outline is jagged (Fig. 5, Series IVB) after protoplasmic setting.

It appears that 'vacuole' formation is not an active secretion of liquid by the protoplast but a direct result of a centrifugal migration of protoplasmic granules. This is clear from the fact that Brownian movement does not decrease throughout the cell uniformly but rather ceases from the edge inwards. The fact that in nature the 'vacuole' appears excentrically, i.e.

the protoplasmic granules conglomerate towards the lumen of the ring, may be connected with the subsequent protoplasmic activity at the site of nematode penetration.

Final setting of the protoplasm may be long delayed after the 'vacuole' has been formed, and it is possible in a heat- or shock-stimulated preparation to observe Brownian movement of the protoplasm for 6 or more hours after 'vacuole' formation.

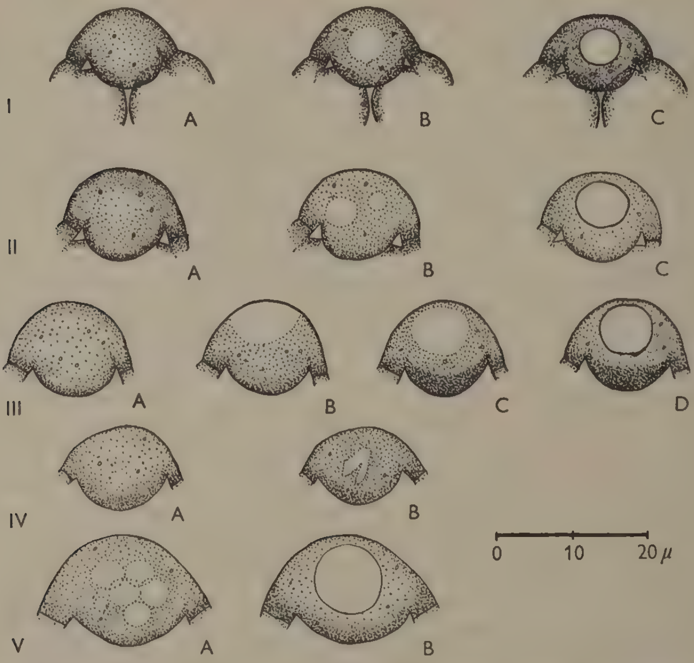


Fig. 5. Post-inflation stages of *D. doedycoides*. Series I, central 'vacuole' formation. Series II, central 'vacuole' formation with 'vacuole' coalescence. Series III, excentric 'vacuole' formation with endocentric 'setting' of protoplasm. Series IV, central 'vacuole' formation with jagged 'vacuolar' outline in B. Series V, as Series II.

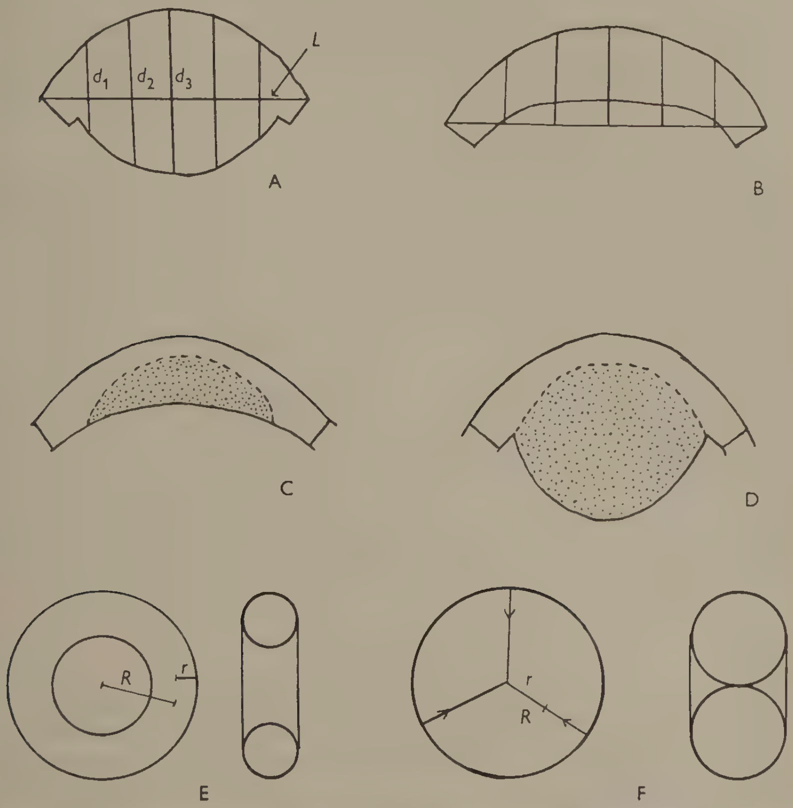
The extent of cell inflation

Comandon & de Fonbrune (1938) stated that the cell volume 'more than triples' when the ring cell inflates, though no information is given as to how this figure for volume increase was arrived at. As any quantitative investigation must be based on the actual volume increase this was measured as follows.

Sixty cells of *D. doedycoides*, half of which were unconstricted and half constricted (hot water, 45° C.), were drawn under oil immersion with the camera lucida. A micrometer scale for the particular magnification was also drawn. The volume was calculated from the formula:

$$V=\tfrac{1}{6}(d_1^2+d_2^2+\dots+d_6^2)\,.\,\tfrac{1}{4}\pi\,.\,L,$$

where V = cell volume in μ^3, L = total length of cell in μ, and d_1-d_6 = mean diameters of six cell sections in μ (Fig. 6A, B). The mean cell volume of the uninflated cell was found to be about 2500 μ^3 and of the inflated cell about 3200 μ^3. The mean increase in cell volume during inflation is thus by a factor of 3·3, so that the estimate of Comandon & de Fonbrune is a good one.



ig. 6. A–D, *D. doedycoides*; E, F, *A. dactyloides*. A, B, inflated and uninflated ring cells showing method of determination of volume (see text). C, D, diagrammatic representation of inflated and uninflated cells showing localized area of plastic flow during inflation. E, F, constricted and unconstricted rings showing alternative method of volume determination (see text).

The calculation introduces a mathematical error which arises from the act that the cell cross-sections are not strictly circular. An alternative nethod was used and applied to rings of *A. dactyloides*. The average dimenions of entire rings were used. These were as follows (Fig. 6E, F).

Unconstricted rings: mean radius $R = 11{\cdot}25\ \mu$, mean radius $r = 3{\cdot}25\ \mu$. Therefore volume of the unconstricted ring is given by

$$2\pi R\pi r^2 = 2\pi^2 . 11{\cdot}25 . (3{\cdot}25)^2.$$

Constricted ring, assuming complete constriction, mean radius $R = 7{\cdot}25\ \mu$, nean radius $r = 7{\cdot}25\ \mu$. Volume: $2\pi R\pi r^2 = 2\pi^2 . 7{\cdot}25 . (7{\cdot}25)^2$.

Increase factor:

$$\frac{(7{\cdot}25)^3}{11{\cdot}25 \times (3{\cdot}25)^2} = \frac{380}{119} = 3{\cdot}2.$$

Thus, this increase factor obtained by a mathematical formula applied to the entire rings of *A. dactyloides* is in close agreement with the result obtained by using the average dimensions of cells of *D. doedycoides*. Though both calculations are approximate, the agreement between them and also with that of Comandon & de Fonbrune establishes some confidence in the methods of estimation.

The osmotic potential of uninflated and inflated cells

The next experiments were designed to determine whether the osmotic potential of ring cells changes on inflation. Normal, unconstricted rings were placed in sucrose solutions of various molar concentrations for about 10 min. Plasmolysis of 50 % of the cells was found to occur in a concentration of 0·6 M. The experiment was repeated a number of times with preparations 6, 7, 10 and 14 days old. The cells did not change in shape and the protoplast invariably retracted from the inner wall of the ring cell (Fig. 4J). Ring cells of the same ages, but first inflated by hot water stimulation showed, on immersion in 0·5 M sucrose, some very slight shrinkage which caused a slight opening of the ring (Fig. 4K). In 0·6 M sucrose, definite plasmolysis occurred in 50 % of the cells. In 0·7 M sucrose, the protoplast floated freely in the cell. In a number of cells the cell wall decreased in area quite considerably, in others not (Fig. 4M, N). Thus inflation does not appreciably alter the osmotic potential of the cells.

Discussion

Before discussing the hypotheses of ring constriction put forward by previous workers, the means by which the process has been investigated here, and the results on which the subsequent arguments depend, will be briefly recapitulated.

First, the sequence, which according to previous workers normally takes about one-tenth of a second, can be slowed down more than 100 times by suitably stimulating the rings immersed in a sucrose solution of appropriate strength, and then slowly diluting the solution, so allowing the response to stimulation to take place gradually. As far as can be seen, the response obtained in this way is a normal one, except in respect of time to completion.

Secondly, the rate of Brownian movement of protoplasmic or other granules within the ring cells is an indication of the viscosity of the protoplasm and cell sap. A decrease in viscosity expresses itself as an increase in the amplitude of the Brownian movement of the granules, and evidence based on this consideration is relevant to the problem. Both Einstein and Smoluchowski (Hatscheck, 1925) have evolved formulae indicating the dependence of Brownian movement of suspended particles on the viscosity of the medium.

Results show approximately a threefold increase in volume of the ring cells on inflation. As the osmotic potentials (equivalent to 0·6 M sucrose) of these cells obtained plasmametrically before and after cell inflation are equal, a threefold increase of osmotically active substances must occur. During inflation regions of low viscosity appear in the inflating cells either outside or inside the main protoplasmic bulk of the cell. These regions are at least initially not separated from the remainder of the protoplasm by a membrane, as protoplasmic granules pass from one region into the other without restraint.

Since inflated cells do not entirely collapse on plasmolysis, inflation is not merely a matter of elastic stretching of the cell wall but a plasto-elastic phenomenon. Deformation of the cell wall during inflation is partly elastic and recoverable, as has been shown by plasmolysis of constricted rings, but it is largely permanent and due to plastic flow. It appears that the wall of a newly inflated cell is more elastic than the wall of one which has been in an inflated state for some time. This would suggest a relatively slow but permanent cell wall 'fixation'. On inflation the cell wall decreases in thickness towards the lumen of the ring, and annular thickenings, perhaps due to micro-fibrillar slip, may on occasion be observed (Fig. 4E). Occasionally but rarely, the internal circular wall of the cell near the transverse septum may appear thickened (Fig. 4D). This same part of the wall retains its shape on cell inflation in almost all instances (Figs. 1G, 4N and 4K). This suggests that, as the cell inflates, an essential difference develops between areas of the wall which, prior to inflation, were morphologically indistinguishable. The parts of the wall which exhibit plastic flow during inflation are strictly localized (Fig. 6C, D).

As would be expected, ring cell protoplasts are surrounded by a membrane as Brownian movement of protoplasmic granules is restrained at this boundary. The plasmolysis experiments have shown that this membrane is present in its entirety before and after inflation, although the volume of the protoplast contained within it increases threefold.

Lastly an osmotic potential of > 0·3 M sucrose will prevent inflation, and thus stimulation and inflation (i.e. the reaction to stimulation) can be separated in time.

In short, ring constriction (cell inflation) involves rapid changes in the volume, the osmotic properties, the cell wall and the plasma membrane structure of the inflating cells. It now remains to consider how these changes may be causally related to the cell inflation process.

Couch (1937) suggested that a re-arrangement of water and colloidal material already in the cell might be responsible for the increase in cell volume. Perhaps he was prompted to this suggestion by the unprecedented rapidity of inflation, and by his erroneous observation that after inflation the ring cell contained a 'doubtless gelatinous or colloidal substance'. However, water or cell sap is, under the conditions of normal cell inflation, incompressible and inexpansible, and it cannot suddenly treble its volume by re-arrangement of molecules. The only instance of which the writer is aware of a molecular volume increase is from an enzymatic study by Eyring, Johnson & Gensler (1946). These workers showed that the unfolding of protein chains is accompanied by 'an extraordinary increase in molecular volume of the order of $\frac{3}{4}$ % per 100 or so amino-acid residues, or 60–70 ml. per mole of protein'. This example thus shows a molecular volume increase of less than 1 %. The volume increase of the ring cells is about 300 % for the specific substance postulated and there is no evidence that such a mechanism exists. Furthermore, as the unfolding of protein molecules is accompanied by an increase of their absorption capacity (*int. al.* Goldacre, 1952) it seems unlikely that liquid vacuoles should appear within the cell during inflation, as sometimes occurs. Thus this 'colloidal re-arrangement' hypothesis is not tenable, and it is entirely inconsistent with our present knowledge of high-polymer mechanics.

Couch's second theory, that 'additional water is imbibed from the stalk or thread cells' is also not borne out by facts. It would mean that on ring-constriction approximately 18,000 μ^3 of water must pass through 2–3 successive stalk-cell septa, each of an area of less than 25 μ^2, in one-tenth of a second. These septa are not visibly perforated. No change or movement of protoplasmic granules has ever been observed by the present writer in the stalk cells during experimentally induced ring-constriction. Furthermore, if Couch is right, water would have to pass to the central ring cell wholly through one, or partly through two other ring cell protoplasts, which are being totally reorganized at the same time. In this connexion it must be emphasized that a central ring-cell can inflate slowly following dilution of the sucrose solution in which it is mounted, without causing any visible disturbance in the contents of the two adjacent ring-cells, and if these also inflate after the central cell has started, this does not delay inflation of the central cell. It is clear, therefore, that the three cells do not compete for available water from the stalk cells, but behave more or less independently.

Comandon & de Fonbrune (1939) suggested that volume increase is due to a modification of the osmotic pressure *or* of the permeability (presumably to water rather than to solutes) of the cell membrane. The latter supposition is unlikely. This 'permeability mechanism' would necessitate a very high suction potential in the uninflated cell, which on a sudden increase in the permeability of the cell membrane would tend to draw water rapidly into the cell. However, such a change in permeability could by itself affect only the *rate* of water uptake, not the final equilibrium attained. In order to change this too, a modification of suction potential *as well as* of permeability would be necessary. The facts that the osmotic potentials of the inflated and uninflated cells are essentially alike, that inflation is stopped by 0·2 M sucrose solution on the outside of the cell and recommences on its dilution, emphasize further that inflation cannot be brought about solely by a change in the permeability of the protoplasmic membrane. That this plays a part in inflation has neither been proved nor disproved; it may well be involved but cannot be the decisive factor. The suggestion that inflation is due to a modification of the osmotic pressure of the cell contents will be discussed subsequently.

In addition to these published hypotheses, two possible mechanisms have been suggested privately to the present writer, as mentioned in the Introduction. The first of these supposes that the increase in cell volume is due to the production inside the cell of gas, so avoiding the necessity for bsorption of a comparatively large volume of water in a very short time. This explanation cannot, however, be valid, since gas bubbles in the cells vould appear black under the microscope; compare for instance the gas hase in the annulus cells of the sporangia of a fern such as *Dryopteris*, or he gas vacuoles in the blue-green algae. No such phenomena appear in he ring cells.

The second suggestion attributes the cell inflation primarily to the cell vall. This, it is supposed, expands in area and bulges outwards, the proto- last decreases in density under the influence of the reduced pressure so aused within the cell. Pressure equilibrium is then later reached by the low diffusion of water into the cell. This hypothesis is partially invalid; ell inflation without water uptake must involve the appearance of a gas hase which, as has been pointed out, never appears. Consequently cell xpansion and inflow of water must be simultaneous. Additionally it is vident from the plasmolysis of fully inflated cells, that early in the inflation rocess the wall is elastic and tends to collapse on plasmolysis. It is only n the later stages of inflation that a permanent change in the shape of the vall is established. Thus it would appear that the wall lacks the sturdiness nd rigidity which would be necessary to enable it to maintain a reduced ressure inside the cell.

It is worth pointing out in this connexion that while no apparent imilarities other than the rate of change of volume with time exist between he ring mechanism and the reaction of *Mimosa pudica* (Weintraub, 1951–2) or the discharge of basidiospores (Ingold, 1953), a certain resemblance an be found in the discharge of nematocysts in Coelenterates (Robson, 953; Picken, 1953). Here the wall of the thread takes an active part n the mechanism of volume increase by a change in micellar spacing f its material; this has been shown by the stimulation of severed threads, pen at both ends. Extension here is regarded as an intrinsic hydra- ion of the dead wall material. It appears unlikely that these facts are pplicable to predacious fungi and that subsequent dehydration of the vall will produce the elastic effect that has been demonstrated in respect f the ring cells when these are immersed in sucrose solution after nflation.

Whether or not the wall exhibits an active part, i.e. an intrinsic expan- ion on cell inflation, could be tested by immersing a ring in 0·5 M sucrose, timulating it, tearing it open and then diluting the medium. If the wall xpands in area under these conditions an active wall mechanism must perate. This experiment was attempted but due to experimental diffi- ulties remained inconclusive.

If, as seems almost certain, the wall plays an entirely passive part in cell nflation, an alternative mechanism must operate. It must then be assumed hat the wall is pushed outwards by pressure from within the cell. The rotoplasmic content is diluted during inflation, as is obvious from its lecrease in viscosity and threefold increase in volume, and as the rings are ntirely surrounded by water during the inflation, water apparently goes nto the cell. This must be caused by a sudden increase in the suction otential of the cell. This can only occur in two ways, either the wall ressure of the cell falls or the osmotic potential of the cell increases. The question is simply which of the two phenomena (or both) are involved and what is their timing in relation to the inflation process.

A first hypothesis might suggest that stimulation induces a rapid increase in the osmotic potential of the ring cells from a value equivalent to 0·6 M sucrose up to one equivalent to 1·8 M. Following this there would be a rapid uptake of water from the surrounding medium. This would probably require an increase in the permeability of the ring cells (i.e. permeability to water, not to solutes) since the rate of change of cell volume is so extremely rapid compared with changes observed in normal plasmolysis. Elastic extension of the wall, followed by plastic flow, leading to a permanent change in its structure and shape, would then take place as the high osmotic potential developed within the cell, so that the uptake need not be seriously restricted by wall pressure. This plastic flow in the wall might be simply a consequence of the mechanical effect of the internal pressure, or might involve some more fundamental enzymatically 'triggered' change in wall structure. If, however, the driving force of the cell inflation is one equivalent to 1·8 M sucrose, then it seems unlikely that 0·2 M sucrose applied externally should inhibit it completely.

It thus seems necessary to adopt a second hypothesis, postulating an alternative mechanism whereby stimulation of the inner wall of the ring cell induces an instantaneous decrease in wall pressure and an increase in membrane permeability in that particular region of the wall. The resultant increase in the suction potential would draw in water from the surrounding medium. As this uptake proceeded, the level of osmotically active substances in the cell would rise, due to hydrolysis of large molecules present there, so maintaining the inflow of water. The osmotic potential would also be maintained at about 0·6 M, never rising much above a level necessary to maintain the plastic extension of the wall. This process would continue until the inflation was complete, when the wall structure became 'fixed'. In this way it would be possible to account for the restrictive effect on cell inflation of externally applied 0·2 M sucrose solution. The osmotic force involved in cell expansion need never exceed this level.

Alternatively, it is possible that the osmotic recovery is rather more slow. The cell would be enabled to expand by virtue of its initial osmotic potential, which falls during expansion from a level equivalent to 0·6 M sucrose to one finally equivalent to 0·2 M, due to water uptake.

The cell would recover its initial osmotic potential after inflation by relatively slow hydrolysis of polymers present within it, and/or by relatively slow transport of solutes from the stalk cells. The inevitable interval between cell-inflation and the plasmametric estimation of its osmotic pressure would allow this recovery of osmotic potential to take place undetected.

Both versions of the last-mentioned hypothesis seem to be consistent with the known facts. Both postulate a change in wall structure, producing a sudden decrease in wall pressure, and an increase in permeability of the cell membrane to water. The amount of osmotically active material in the cell then trebles but the rate at which this happens and its timing in relation to the inflation process is as yet unknown. It is in this point that the two versions of the hypothesis differ fundamentally. If the rate of ncrease of osmotically active substances in the cell is very fast it seems ikely that intracellular hydrolysis of large molecules occurs. If it is slow, transport of these substances into the cell may account for it.

It will be noted that the second version of this hypothesis is the less physiologically exacting. It envisages stimulation of the cell as inducing a change in wall structure and a change in permeability, but does not require in addition any exceptionally rapid increase in the amount of osmotically active solutes in the cell. On the reasonable assumption that the stimulus-sensitive part of the cell wall has some rather special structure, n which perhaps the protoplast and the cell wall are abnormally intimately nter-related, changes in wall structure and membrane permeability may well be similarly interconnected and may thus reasonably be expected to be induced by the same stimulus. Hydrolysis of cell polymers might, on the other hand, be expected to involve some rather different centres of activity in the cell.

Under normal, natural conditions in which ring constriction results in the capture of a nematode, it is followed after a certain time interval by growth of hyphae from the ring cells into the tissues of the prey. This growth must inevitably involve transport of solutes from the parent hypha nto the ring cells, and it is thus not unreasonable to regard their post-nflation recovery of osmotic potential as taking place in this way; it is an essential preliminary to the final exploitation of the capture.

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NEW AQUATIC HYPHOMYCETES: *LEMONNIERA BRACHYCLADIA*, *ANGUILLOSPORA CRASSA* AND *FLUMINISPORA OVALIS*

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(With Plates 20 and 21 and 5 Text-figures)

An illustrated account is given of three freshwater aquatic hyphomycetes: *Lemonniera brachycladia* sp.nov., *Anguillospora crassa* sp.nov. and *Fluminispora ovalis* gen.nov., sp.nov. Spores belonging probably to an undescribed species of *Triscelophorus* are also illustrated.

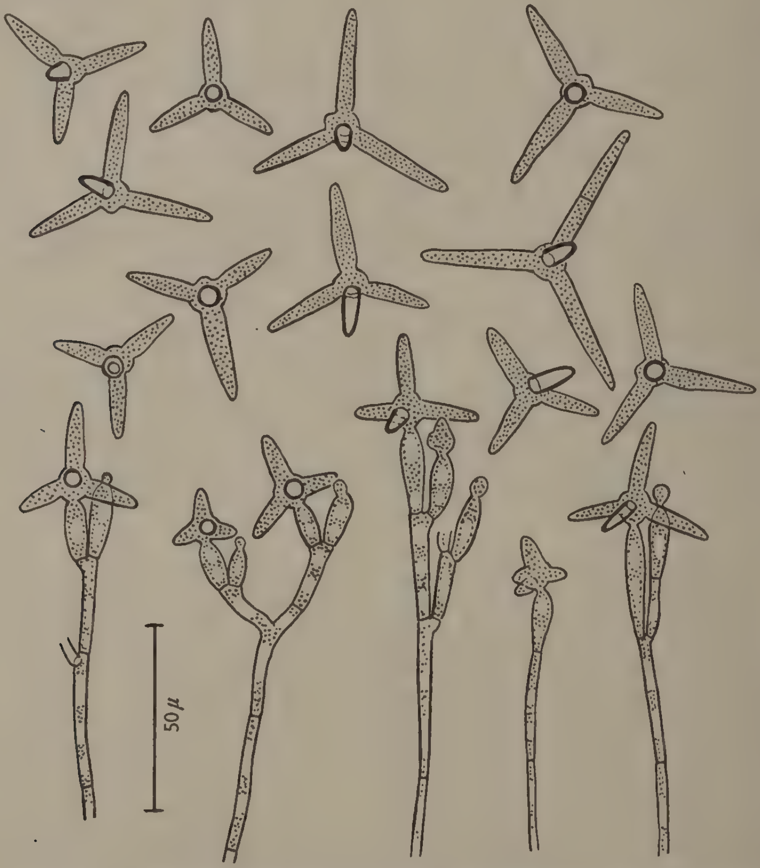
Lemonniera brachycladia sp.nov. (Pl. 20, Text-fig. 1)

ungus aquaticus submersus, mycelio repente septato, ramoso, primum hyalino, postea runneo. Conidiophora hyalina, septata, in unam aut plures phialides finiens. Conidium hialospora) hyalinum, ex quattuor aequalibus divergentibus bracchiis a media primo-enita parte orientibus. Unum quodque bracchium 15–50 μ longum, 4–6 μ latum prope asim ad apicem 3 μ attenuatur, plerumque aseptatum, raro uniseptatum. *Hab.* In foliis utrescentibus *Alni glutinosae* etc. in rivis jacentibus, Ide Hill, Kent, Anglia. Herb. I.M.I. 810.

Lemonniera aquatica de Wild. is an exceedingly common aquatic hypho-ycete found in almost every collection of submerged decaying leaves hich I have examined from streams in Britain and Ireland (Ingold, 943 *a*). It is also known from continental Europe (de Wildeman, 1894; ngold, 1949) and from California, U.S.A. (Ranzoni, 1953). From there anzoni has described a second species, *L. cornuta*. In *L. aquatica* one of e four divergent arms of the spore is in line with the parent phialide, but *L. cornuta* this is not so.

For some years I have been aware that on submerged decaying leaves species of *Lemonniera* sometimes occurs which, though close to *L. aquatica*, iffers from it in a number of small, but apparently constant, characters. urther, spores of this fungus have been seen in many samples of stream um. I have refrained, however, from its description until it could be udied in pure culture. Recently an isolate was made from a spore picked ut of scum, and the behaviour of this has confirmed the view that a istinct species is involved.

In *L. brachycladia* (Text-fig. 1 and Pl. 20) the agreement with *L. aquatica* close. As in that species one of the four arms of the spore is in line with e phialide that bears it. However, in *L. brachycladia* the arms of the ature spore are shorter and stouter than in *L. aquatica*. Further, whereas *L. aquatica* the sides of each spore-arm are nearly parallel, in *L. brachy-adia* they distinctly converge towards the free ends. In the genus *Lemon-era* the spore starts as a spherical primordium from which the four divergent arms grow out simultaneously. In *L. aquatica*, as the spor matures, the spherical outline of the original primordium is usually completely lost. However, in *L. brachycladia* traces of outline of the centra region are generally retained in the mature spore. Finally in *L. aquatic* there is a greater tendency to septation in the arms of the spore than i *L. brachycladia*.



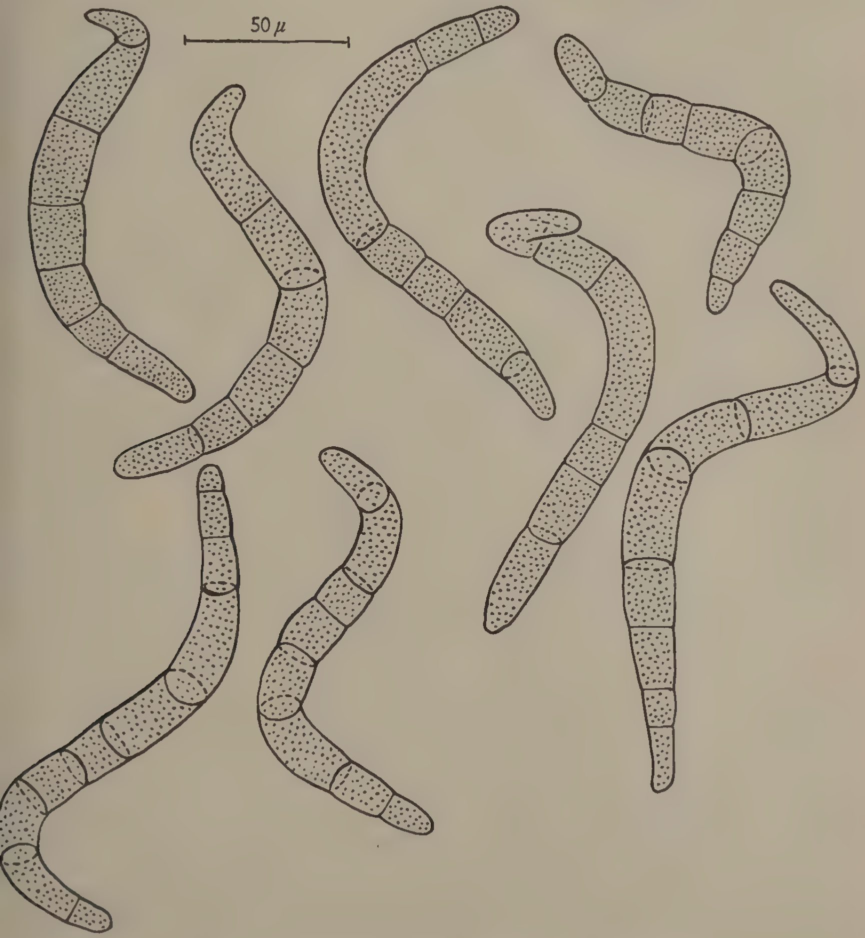
Text-fig. 1. *Lemonniera brachycladia* conidia and conidiophores formed from pieces of agar culture in aerated water.

A colony on 2% malt agar grown for 28 days at 20–22° C. had diameter of 1·8 cm. It was very compact with aerial growth less tha 0·5 mm. high. The margin was white, but the older parts were chestnu brown due to hyphae, many of which were swollen, with brown walls This mass of brown hyphae had almost a sclerotial consistency. The colon closely resembled that of *L. aquatica*. No spores were produced in a ordinary Petri dish culture. However, when strips of colony were sub merged in water and strongly aerated, sparse sporulation occurred afte 2 days. After only a few weeks in culture the isolate appeared to lose it capacity to form spores even under these conditions.

Anguillospora crassa sp.nov. (Text-figs. 2, 3)

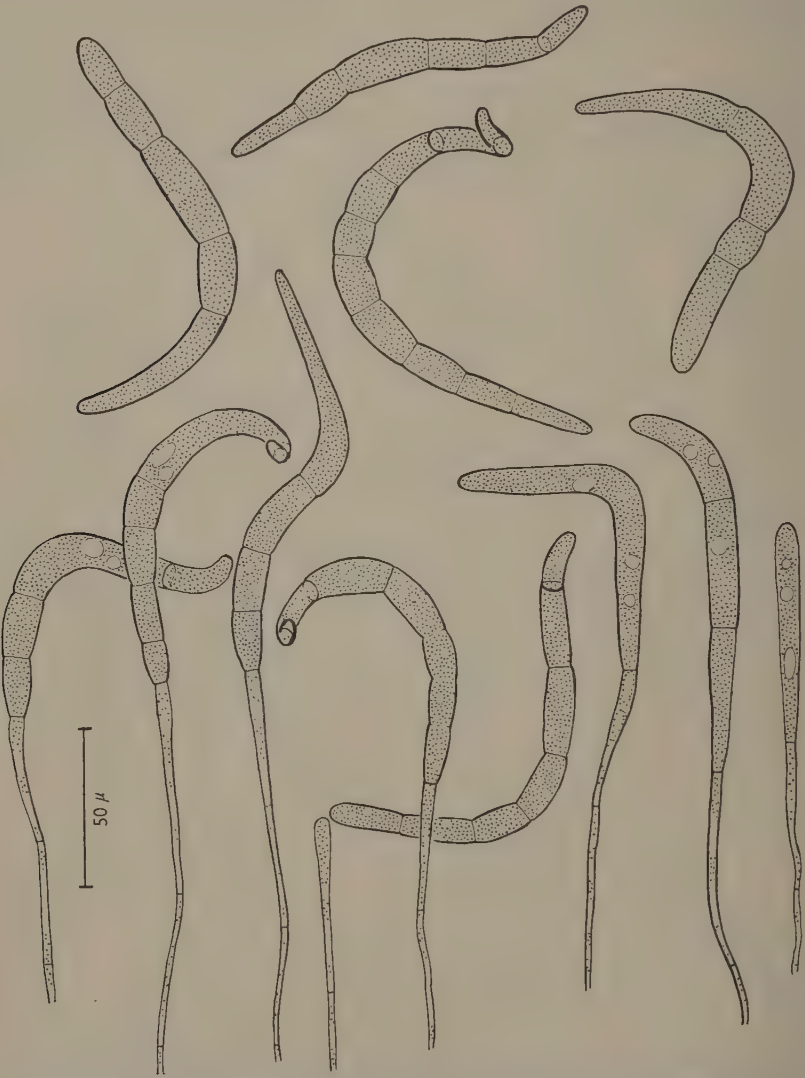
ungus aquaticus submersus, mycelio repente septato, ramoso, primum hyalino, postea livaceo-fuscescente. Conidiophora hyalina, simplex, unum conidium terminale gerens. onidium (thallospora terminalis) hyalinum, sigmoides, 5–10-septatum, 120–200 μ ngum, 15–20 μ latum in media parte utrinque ad 8–10 μ attenuatum. *Hab.* Ex rivo d Ide Hill, Kent, Anglia. Substratum incognitum. Herb. I.M.I. 70811.

For some years past I have frequently examined scum from an alder-ned stream between Ide Hill and Toys Hill in Kent. In the autumn and inter this scum always contains a rich spora of the commoner aquatic



Text-fig. 2. *Anguillospora crassa.* Spores from stream scum.

phomycetes, but it is noteworthy for the abundance of certain spores hich are not, in my experience, so common elsewhere in Britain. mongst these may be mentioned those of *Actinospora megalospora*, some autiful pearly 4- or 5-radiate spores clearly belonging to an undescribed genus (Ingold, 1942, fig. 1, no. 17), and long, fat, sigmoid spores. Recently a pure culture, derived from a spore of this last type, was made and, as suspected, the fungus is referable to *Anguillospora.*



Text-fig. 3. *Anguillospora crassa.* Conidia and conidiophores formed from pieces of agar culture in aerated water.

The type species of this genus, *A. longissima* (Ingold, 1942), is abundant. Another species, *A. flagellifera* (Ingold, 1949), found on a submerged decaying leaf from a stream in Switzerland, should certainly not have been assigned to that genus. It is clearly a species of *Centrospora* and, indeed, the Swiss collection could probably have been identified as *C. acerina.*

Ranzoni (1953) described two new species, *A. macrospora* and *A. pseudolongissima*. Both differed from the type species in the mode of spore liberation. In *Anguillospora* the spore is very long, septate, and S- or L-shaped with the letter lying in more than one plane. The spore is simply the specialized, glycogen-enriched end of a hypha. There is no sudden change in width on passing from the unspecialized conidiophore to the conidium. In *A. longissima*, as the conidium ripens, a small cell differentiates at the end of the conidiophore and it is by the breakdown of this that the spore is set free. However, in *A. macrospora* and *A. pseudolongissima* liberation is by disarticulation at the cross-wall separating the conidium from the conidiophore. I agree with Ranzoni in placing his two species in this genus in spite of the method of spore liberation. In *A. crassa* the spore is set free in the same manner. It is, however, sharply distinguished from Ranzoni's species by its very large size.

Naturally occurring spores from stream scum (Text-fig. 2) are hyaline, S- or L-shaped, 120–200 μ long and 15–20 μ wide in the middle region tapering to 8–10 μ at the ends. They are pearly due to abundant glycogen in the cells. A colony grown in the dark at 20–22° C. on 2 % malt agar for 4 weeks had a diameter of 30 cm. It was compact with felt-like aerial growth less than 1 mm. high. The central region was greenish grey, but there was a white margin several millimetres in width.

No spores were produced on the agar culture, but if strips of the colony were submerged in water through which a vigorous stream of air was bubbled, abundant spore production occurred in a few days. The spores produced agreed fairly well with those seen in nature but they tended to be more variable in length and to have a somewhat reduced width (Text-fig. 3).

Fluminispora gen.nov.

Fungi aquatici submersi, mycelio repente septato, ramoso, hyalino. Conidiophora hyalina, ramosa. Conidium unicellulare, hyalinum, ovale aut subreniforme. Conidium quod primum oritur terminale est, constrictum qua cum conidiophora jungitur. Conidia quae postea oriuntur, prope priora conidia ab altero latere apparent. Typus:

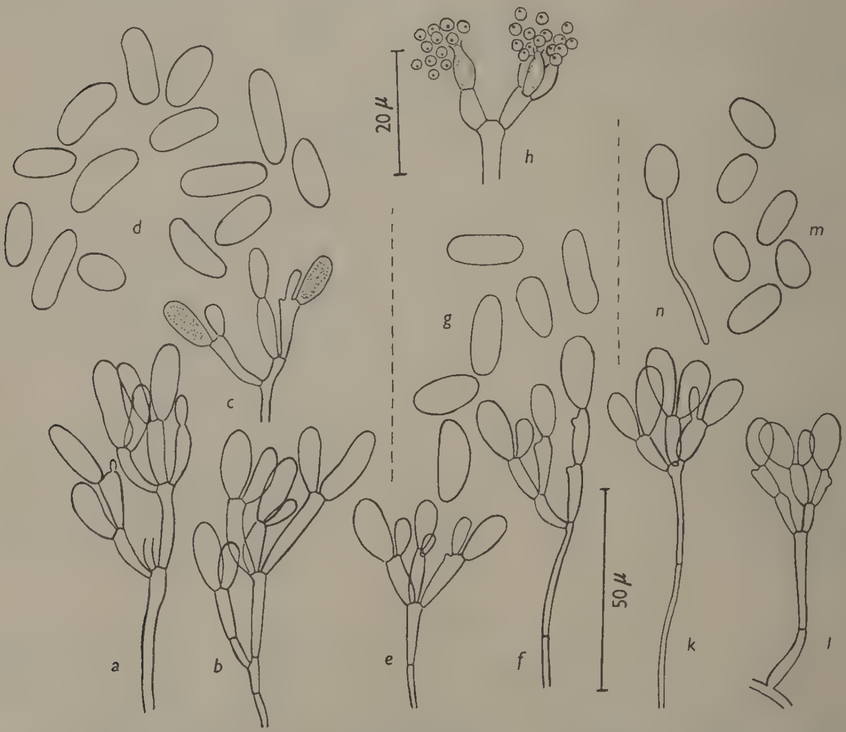
Fluminispora ovalis sp.nov. (Pl. 21, Text-fig. 4)

Fungus aquaticus submersus, mycelio repente septato, ramoso, hyalino. Conidiophora hyalina, ramosa. Conidium (thallospora) hyalinum, unicellulare, ovale aut subreniforme, glycogen continens, 10–22 μ longum, 6–8 μ latum. Conidium quod primum oritur terminale est, constrictum qua cum conidiophora jungitur. Conidia quae postea oriuntur prope priora conidia ab altero latere apparent. Saepe duo (raro tria) conidia conidiophorae conjuncta adsunt in fine unius cuiusque rami conidiophorae. Phialospora hyalina globosa, 1·5–2 μ dia., in culturis solum visa. *Hab*. In foliis putrescentibus *Alni glutinosae* in rivis acentibus, Kingsclere, Hampshire, Anglia. Herb. I.M.I. 70809.

This fungus has been found repeatedly on submerged decaying leaves of deciduous trees from streams at Kingsclere, Hants, Reading, Berks., Sevenoaks, Kent, and Englefield Green, Surrey. It has been isolated in pure culture from all these localities and the different isolates show very close agreement. The description given here is based largely on the behaviour of the organism in culture.

On 2 % malt agar a colony is formed reaching a diameter of 2·5 cm. in

9 days at 20–22° C. The colony is compact, whitish but with a slight creamy tinge. Aerial growth is less than 1 mm. high. For the first 8–10 days a culture forms no spores, but if at this stage small pieces are cut out and strongly aerated in water for 2 days, conidiophores are produced in abundance. In older cultures conidiophores are formed without submergence, but these are normally recumbent on the agar.



Text-fig. 4. *Fluminispora ovalis*. *a–d*, Sevenoaks isolate: *a–c*, conidiophores (*c* shows glycogen in ripe spores dotted); *d*, conidia; *e–h*, Kingsclere isolate: *e* and *f*, conidiophores; *g*, conidia; *h*, phialides and phialospores (contents shown in two phialides); *k–n*, Englefield Green isolate: *k* and *l*, conidiophores; *m*, conidia; *n*, germinating conidium. Conidiophores obtained in all cases by submerging pieces of pure culture in aerated water. All drawn to same scale except *h*.

The submerged conidiophore is derived from a hypha which grows out into water from the vegetative mycelium in the agar. Near its free end the hypha usually branches to produce a number of conidium-bearing cells which become somewhat swollen (Text-fig. 4 and Pl. 21). A unicellular, oval, hyaline conidium buds off terminally from one of these cells. Before it is fully mature another usually begins to form close to it. In submerged conidiophores, ripe spores are set free and soon drift away, but in conidiophores on agar they accumulate as a damp mass around the apex of the conidiophore. In submerged cultures rarely more than two spores, often at distinctly different stages of development, are to be seen at the end of each conidium-bearing cell. A study of spore development clearly shows that conidium is a thallospore, but only the first-formed spore on each branch of the conidiophore is truly terminal.

The conidia are mostly oval but a few are subreniform. All are unicellular and contain abundant glycogen. They show considerable range in size: 10–22 $\mu \times 6$–8 μ. The conidium germinates readily in water with a single narrow germ-tube.

In addition to producing these relatively large spores, very much smaller microconidia (phialospores) are formed from typical phialides. These have never been observed on the submerged leaves but only in pure culture. With one exception, all isolates have produced phialospores, although they vary in the freedom with which these spores are formed.

The conidiophore bearing microconidia is simple below but branches towards its free end to give a tight group of rather stout phialides (Text-fig. 4). From each of these minute (2 μ diam.), spherical, hyaline phialospores, each with a single refractive particle, are produced in succession. As with the ordinary conidiophores, those with microconidia can be formed either directly on agar in ageing cultures, or when pieces of colony are submerged in water. Not infrequently the two types of conidia are to be seen on different branches of the same conidiophore.

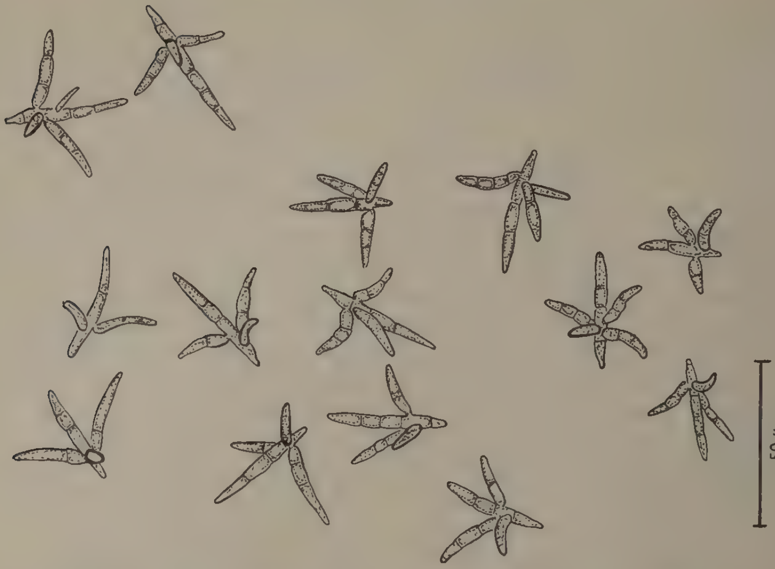
The microconidia in agar cultures form a sticky mass. When formation occurs below water they normally drift away, although sometimes short chains of two to four spores may be seen at the end of a phialide. All attempts at germination have failed.

Although I have known this fungus for over 10 years and have isolated it in pure culture again and again, I have refrained from describing it partly because its spore is not morphologically interesting, but mainly because its systematic position is obscure. There are so many genera in hyphomycetes with hyaline, unicellular, oval spores that it would seem this fungus should find a place in one of them. However, my search for a suitable genus has failed and two distinguished authorities on hyphomycetes whom I consulted have no suitable suggestions to offer. I have, therefore, reluctantly described it as the type of a new genus.

A possible Triscelophous *species*

During the autumn of 1956 I encountered spores in scum from a stream running into Virginia Water, Surrey, which were new to me (Text-fig. 5). It is not proposed to erect a species on the basis of these, but the production of this paper affords an opportunity of recording their occurrence. The spore is hyaline, consisting of a main axis with normally three laterals arising near its base. This structure would suggest an undescribed species of *Triscelophorus* at present represented by a single species, *T. monosporus* (Ingold, 1943*b*).

Since this paper was drafted all the new species described here have been seen during the recent Foray of the Society at Dublin: spores of *Lemonniera brachycladia* from the Dargle Glen near Bray; spores of *Anguillospora crassa* from the Dargle and also from a stream at Townley Hall, Co. Louth, and *Fluminispora ovalis* growing on submerged decaying alder leaves from the Dargle Glen.



Text-fig. 5. Spores of a possible *Triscelophorus* sp. from Virginia Water, Surrey.

My thanks are due to Vivienne Dring who made most of the isolations on which the paper is largely based and to Prof. E. H. Warmington for his invaluable help with the Latin diagnoses.

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EXPLANATION OF PLATES 20 AND 21

Plate 20

Lemonniera aquatica top left; remainder *L. brachycladia*. In the middle picture one of the phialides has grown out into an ordinary hypha.

Plate 21

Fluminispora ovalis. *Top:* normal conidia (thallospores) and phialospores from Kingsclere isolate. *Middle:* three conidiophores of Englefield Green isolate; that on the right is young and each branch bears only its first-formed spore. Photographed from living material. The background has been blocked out. *Bottom:* two conidiophores (Kingsclere isolate) stained in iodine.

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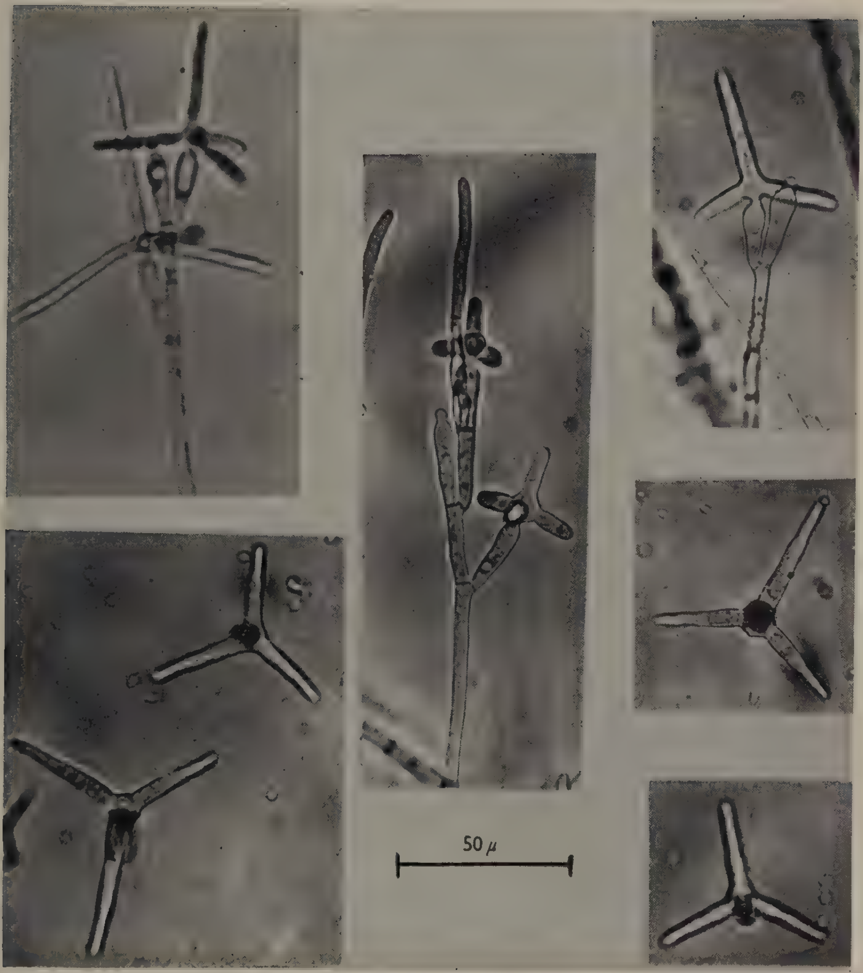
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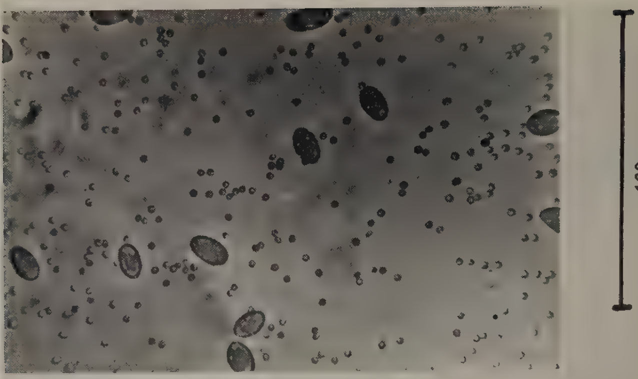
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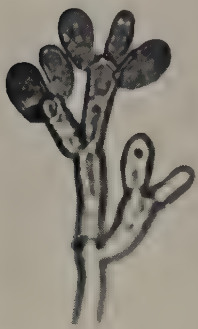

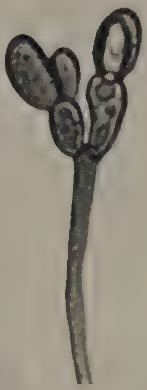

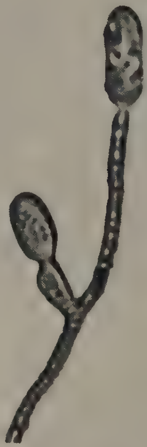

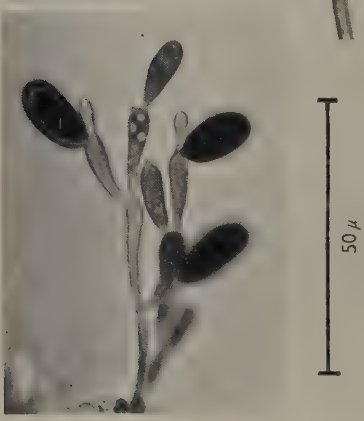
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Trans. Brit. mycol. Soc. **41** (3), 373–384 (1958).

PHYSIOLOGICAL SPECIALIZATION OF OAT CROWN RUST (*PUCCINIA CORONATA* CORDA F. SP. *AVENAE* (ERIKSS. & HENN.) ERIKSS.)

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The investigations described concern physiological differentiation in crown rust of oats (*Puccinia coronata* Corda f. sp. *avenae*). The pathogenic reactions of thirteen races identified from twenty-four uredospore collections are presented, and the results discussed in relation to the problem of breeding disease resistant cultivars of oats in Britain.

Introduction

A considerable amount of work has been carried out in Europe and North America on the various aspects of specialization in *Puccinia coronata* Corda since Eriksson (1894), and Eriksson & Henning (1894), first drew attention to the fact that pathogenic fungi varied in their types of host range.

In Europe, Klebahn (1892) first divided *P. coronata* into two species, *P. coronata* (Corda) Kleb. and *P. coronifera* Kleb. No clear morphological differences could be observed between these two species, their subdivision being based upon their ability or failure to infect the two alternate hosts, *Rhamnus frangula* L. and *R. cathartica* L. *P. coronata* had its aecidial stage on *R. frangula* and *P. coronifera* on *R. cathartica*. As a result of further work by Klebahn (1895, 1896, 1898, 1912), Eriksson (1894, 1897, 1909) and Mühlethaler (1910, 1911), these two species were further divided into *formae speciales* on their restriction of infection to certain uredial host species. They differentiated five specialized forms of *P. coronata*, and nine forms of *P. coronifera*, the latter including f. sp. *avenae* on oats.

Frenzel (1930), and other German investigators, have recognized Klebahn's classification, but the justification for the subdivision of the morphological species into two species merely on the basis of its aecidial host reaction was questioned by several workers (Melhus, Dietz & Willey, 1922; Dietz, 1926; Fraser & Ledingham, 1933; Murphy, 1935; Straib, 1937; Brown, 1938), who, in consequence, retained all the *formae speciales* (varieties, parasitic races) distinguished by them under the original species *P. coronata* Corda. Both Melhus *et al.* (1922) and Fraser & Ledingham (1933) showed that certain species of *Rhamnus* acted as differential hosts for the various *formae speciales* of *P. coronata* in the same manner as cultivars and species of *Avena* serve as differential hosts for physiologic races within a varietal form such as *P. coronata* f. sp. *avenae* (Murphy, 1935).

The existence of physiologic races within f. sp. *avenae* which could only be determined by differences in their ability to infect certain cultivars or species of oats was first demonstrated by Hoerner (1919). He distinguished four physiologic forms of the rust on the basis of differential reactions on the two host cultivars, Green Russian and Ruakura Rustproof and his data indicated that more races might have been recognized by using a larger number of cultivars as differential hosts.

Through the use of extended and more efficient sets of differentials, investigations in many countries since then have produced evidence of extensive specialization in the oat crown rust pathogen.

The importance of selecting an efficient standardized set of differential host cultivars for purposes of comparing the pathogenic characteristics of the races identified was soon realized. All the physiologic forms described and reported up to 1934 in North America were compared and given standardized numerical designations by Murphy (1935) on the basis of their reaction on eleven selected host cultivars, which, together with the cultivars Bond and Victoria, became accepted as the standard set of differentials for race identification work.

In a report published recently by Simons & Murphy (1955), details are given of the average reactions of this series of hosts to no less than 113 distinctive races which have been identified to date by this means, as well as an additional fifty-nine races identified through the use of the revised set of ten differentials introduced in 1951 (Simons & Murphy, 1952).

Whilst American workers recognized only clear-cut differences in host reaction for purposes of race differentiation, the two European investigators, Frenzel (1930) and Straib (1937) utilized finer distinctions in determining races. For example, Frenzel considered that minor differences such as those between 0-type and 1-type reactions, both of which are classed as resistant, were sufficient to differentiate physiologic forms. As a result both workers have reported relatively greater pathogenic variability in their rust collections than have the American investigators. Frenzel distinguished thirty-three separate races in twenty-seven collections made in Germany during 1928 and 1929 on the basis of their reactions on nine cultivars. In 1935 and 1936, Straib (1937) tested 147 collections from different locations in Germany on fifteen cultivars and distinguished 142 races. His results were similar to those of Frenzel in that he distinguished a new and distinct race in nearly every collection through the recognition of small differences in degrees of resistance or susceptibility.

In Britain, very little work appears to have been done either on physiologic specialization in this pathogen or on the reaction of the more widely grown cultivars of oats to the recognized races. Brown (1937) mentioned the occurrence of three physiological races (designated 6, 42 and 44) and observed their pathogenicity on three fairly widely used cultivars, and, more recently, Williams & Verma (1956) reported on the reactions of various forms of the genus *Avena* to three cultures, one of which (designated by them as A) was supplied by the writer.

In the absence of any available information on the pathogenicity of the biologic forms prevailing in Wales, a study of this aspect was considered desirable as part of the breeding work undertaken with the view of developing crown rust resistant cultivars adapted to British conditions.

The information obtained on physiologic specialization in the course of these investigations is presented here and discussed in relation to the problem of breeding resistant cultivars of oats.

MATERIALS AND METHODS

Uredospore collections of crown rust obtained from several different sources in Wales and south-west England were cultured separately in the first instance on seedlings of the susceptible winter cultivar of oats S. 172. From these, representative single spore cultures were then established by means of the technique adopted by Newton & Johnson (1932) for *P. graminis tritici*. The spores were scattered sparsely over a sterile slide, observed through the low power of a microscope, picked up separately on the point of a fine needle and transferred to the leaf surfaces. It was necessary to make a number of single spore inoculations from each collection, since even under optimum conditions less than 20 % caused infection.

All inoculations were carried out during the winter in a heated glass-house where the daily temperature ranged from 58° to 68° F. Owing to the difficulty of keeping the glass-house cool in summer, the actual testing for seedling reaction was largely confined to the cooler months of the year. Under these conditions pustules generally appeared 10–12 days after inoculations in April, May and September, and after 12–15 days in February, March and October, depending on the prevailing weather conditions.

All plants were sown, grown and prepared for inoculation in a separate glasshouse, and special precautions were taken to prevent contamination. Uninoculated plants placed at random as checks throughout the glass-house showed that, with the maintenance of fairly high humidity, little contamination resulted through air-borne spores. At the end of the incubation period the plants were covered with spore-proof transparent plastic cases, as an additional precaution for preventing contamination of cultures.

Seedlings used to establish pure cultures, or to be observed for their reaction to infection, were inoculated in the first leaf stage. In the physiologic race identification tests, ten or more seedlings of each differential cultivar were inoculated and,to obtain more reliable reactions, tests conducted in the autumn were repeated during the spring when light conditions proved more satisfactory.

In the first place Murphy's standard set of thirteen differentials was used to establish pathogenic differences between the various isolates examined, but since the revised set of ten differentials was introduced in 1951 the different single-spore cultures established from the 1951–53 collections were also tested on the new set. The two sets of standard differential hosts used comprised the cultivars shown in Table 1.

The reactions of the different cultivars to infection by different cultures were classified according to the scheme drawn up originally by Stakman & Levine (1922) for the identification of physiologic races of *P. graminis tritici*, and which with modifications has been adopted by Murphy (1935) and Brown (1937) for *P. coronata avenae*.

In recording observations the five infection classes shown in Table 2, designated by the symbols 0 to 4, were recognized.

In some cases a further refinement of the scale was obtained by attaching plus (+) or minus (−) values to the various classes to indicate heavier or lighter degrees of infection. The *X*- or *M*-type of infection, in which a combination of two or more extreme types occur in varying proportions on the same leaf, was not recognized in these experiments.

Table 1

Series of thirteen differential host cultivars		Revised series of ten differential host cultivars	
Cultivars	U.S.D.A. accession no. C.I.	Cultivars	U.S.D.A. accession no. C.I.
Ruakura	2025	Anthony	2143
Green Russian	2890	Victoria	2401
Hawkeye	2464	Appler	1815
Anthony	2143	Bond	2733
Sunrise	982	Landhafer	3522
Victoria	2401	Santa Fé	4519
Green Mountain	1892	Ukraine	3259
White Tartar	551	Trispernia	4009
Appler	1815	Bondvic	5401
Sterisel	2981	Saia	4639
Belar	2760		
Bond	2733		
Glabrota	2633		

Table 2

Host reaction	Symbol and infection types
Highly resistant	0 = No uredospore pustules developed; necrotic or chlorotic flecks usually present
Resistant	1 = Pustules few, minute and isolated, usually accompanied by pronounced necrosis; necrotic areas often produced without development of pustules
Moderately resistant	2 = Pustules small to medium in size, and surrounded by necrotic or markedly chlorotic areas
Moderately susceptible	3 = Pustules fairly abundant, medium sized and surrounded by chlorotic areas; necrotic areas entirely absent
Very susceptible	4 = Pustules large and abundant, often confluent and showing copious production of uredospores; no necrosis and usually no chlorosis

A first reading was made when primary uredospore pustules were fully developed, usually from the tenth to the twelfth day following inoculation, and the type of infection was recorded. A second reading was taken 4 days afterwards, and occasionally a third a few days later. This was necessary because pustules often did not form on moderately resistant hosts until several days after they had appeared on those which were very susceptible.

Despite all possible efforts to carry out the tests under conditions as near as practicable to the optimum for development of both host and pathogen, a slight fluctuation within an infection type was observed in some cases in the 0, 1 and 2 classes.

PHYSIOLOGIC RACES ISOLATED

Over a period of five years (1949–53), ninety-two single spore cultures from twenty-four different collections were established and examined; twelve of these were made in 1949 and 1950. Except for the collection derived from the breeding nurseries, all were from commercial oat crops grown on the Station's farms, at other centres in the vicinity of Aberystwyth and in South Wales and Cornwall.

On the basis of the reaction of the standard set of thirteen differentials to representative single-spore cultures established from the twelve collections made in 1949 and 1950, five new physiologic races not hitherto described were isolated. These were assigned race numbers 96–100 in accordance with the numerical scheme of nomenclature instituted by United States and Canadian investigators (Simons & Murphy, 1955).

These five different races were distinguishable from one another by the reaction of the seven cultivars: Green Russian, Anthony, Sunrise, Green Mountain, White Tartar, Appler and Sterisel. The behaviour of the other cultivars was similar, or nearly similar, giving susceptible or resistant reactions to each of the five races tested, and consequently was of no assistance in establishing racial differences within this particular group. Ruakura, Belar and Glabrota gave susceptible reactions, while Hawkeye, Victoria and Bond gave resistant reactions to all five races.

Table 3. *Reaction of seven cultivars to physiologic races* 96–100

Differential host cultivars	Physiologic race number				
	96	97	98	99	100
Green Russian	4	4	4	3	0
Anthony	0	0	0–1	4	0
Sunrise	4	4	4	0	4
Green Mountain	0	4	4	4	4
White Tartar	4	4	4	4	0
Appler	0	4	4	4	4
Sterisel	0	0	4	0	0

It will be seen from the reactions of the seven host cultivars presented in Table 3 that, with the exception of races 97 and 98, all these physiologic races differed from one another in their pathogenic reaction on at least two cultivars, thus justifying differentiation into distinct physiologic forms. Differences such as those between 0 and 1 type reactions, and between 3 and 4 type reactions were also observed, but were considered insufficient to justify further subdivision.

During 1951, 1952 and early 1953 collections taken from approximately the same regions were examined. Three single-spore cultures were established from each and their behaviour observed on both the standard set of thirteen cultivars and the revised set of ten.

Twelve physiologic races were isolated by their reaction on the standard set, three of which (races 96, 98 and 99) had been isolated from the 1949 and 1950 collections. One form proved to have identical reactions with race 108 already described elsewhere (Williams & Verma, 1956). When tested on the revised set of differentials, two of the remaining eight forms proved indistinguishable from one another, both giving identical reactions with race 229 already characterized by Peterson in Canada. One other race, namely 232, had also been identified in Canadian collections by Peterson (Simons & Murphy, 1955).

The remaining five isolates here established proved to be new races, and were assigned race numbers 271, 272, 273, 274 and 275, the official race numbers given in accordance with the numerical scheme of nomenclature instituted by North American investigators, and based on reaction to the revised list of differentials.

The infection types produced by the eight forms on the revised set are given in Table 4, and the distribution of races in relation to the individual collections is given in Table 5.

Table 4. *Infection types produced on the revised set of ten standard differential oat cultivars by races* 229 (*a*) (*b*), 232 *and* 271–275 *inclusive*

Differential host cultivar	Physiologic race number 229 (*a*) (*b*)	232	271	272	273	274	275
Anthony	0–1	4	4	0–1	2–3	0–1	0–1
Victoria	0–1	0–1	0–1	0–1	0–1	4	0–1
Appler	1	0	2	3	3	4	3–4
Bond	0	0	0	0	0	4	4
Landhafer	0	0–1	0	0–1	1	0–1	0–1
Santa Fé	0–1	0–1	0–1	0–1	1–2	0–1	0–1
Ukraine	4	4	4	4	4	4	4
Trispernia	0	0–1	2–3	1–2	3	2	1
Bondvic	0–1	1–2	0	1	0–1	2	1
Saia	3	4	4	4	3	0–1	4

Table 5. *Distribution of races in collections* 13–24 *made in* 1951 *and* 1952

Collection no.	Source of collection	No. single spore cultures examined	Physiologic races identified
13	W.P.B.S.:* commercial crop of D. 1670 oats	3	96
14	W.P.B.S.: commercial crop of S. 84 oats	3	96
15	W.P.B.S.: field nursery and variety trials	3	98, 99, 271
16	Cardiganshire: commercial crop of S. 84	3	108
17	Cardiganshire: 1000 ft. above sea-level on Scotch Potato oats	3	232
18	Glamorganshire: unnamed cultivar	3	229 (*a*)
19	Carmarthenshire: crop of S. 84	3	229 (*b*)
20	Carmarthenshire: crop of Onward oats	3	273
21	Cornwall	3	272
22	Cornwall	3	98
23	W.P.B.S.: field nursery on Victoria segregates	4	274
24	W.P.B.S.: field nursery on Bond segregates	4	275

* W.P.B.S. = Welsh Plant Breeding Station.

The most important feature of this survey of the second set of collections was the identification of races 274 and 275, which attacked cultivars that hitherto had proved resistant to all races. Up to July 1952 both Bond and Victoria had proved resistant to all races known in Britain, but in July of that year all cultivars and selections possessing the resistance factors of Bond and Victoria succumbed to attack just before maturity: collections of uredospores from the field nursery revealed the existence of the two new races, 274 and 275, which had not been isolated previously. Bond and its derivatives proved susceptible to both, while Victoria and its derivatives were susceptible to race 274 but resistant to race 275.

Thus, from a total of twenty-four collections made over a period of 5 years (1949–53), no less than thirteen physiologic races were identified and all have given pathogenic reactions different from those of the three races described and isolated by Brown (1937), and from those of cultures *B* and *C* of Williams & Verma (1956).

DISCUSSION

It has long been known that within species of many fungi there are forms or races that are morphologically indistinguishable, but differ from one another in one or more physiological characteristics. Economically the most important of these characters is pathogenicity, and as a result most of the recent workers on specialization in the cereal rust fungi have concentrated on pathogenic specialization and the ability of these organisms to infect different cultivars of the host species.

It is evident from the data obtained during this investigation, and from work already published, that physiological specialization in *P. coronata avenae* is highly developed. It is reasonable to assume that a similar degree of specialization exists in other varietal forms of *P. coronata* Corda, but since they either occur on host species of less economic importance than oats, or cause relatively little damage to their respective hosts, little or no attention has yet been paid to physiologic specialization in these other forms.

Although thirteen physiologic races of *P. coronata avenae* were isolated from twenty-four collections examined during the period 1949–53, probably many more were present. Additional differential cultivars might have been selected that would have made possible the determination of other forms. For instance, two single-spore cultures (races 229 (*a*) and (*b*)) which proved separable on the original set of thirteen differentials, were indistinguishable on the basis of their reaction to the revised set of ten.

The differential host cultivars used for race identification have been arbitrarily selected, and although a more comprehensive set of differentials usually results in the identification of more races than a set comprising fewer cultivars, the latter may prove relatively more efficient than the former. To select an efficient and manageable set of differential cultivars it is necessary to determine the genetic constitution of all those used. Apart from the four cultivars Bond, Landhafer, Santa Fé and Victoria, little is known of the genetic constitution of the other differential cultivars. Without this information their capabilities as differentials when used as a group in combination with each other cannot be established. Theoretically, the maximum number of races that could be determined with a set of ten cultivars would be 2^{10}.

As the number of differential cultivars is increased there is an almost limitless possibility of subdividing what are at present termed physiologic races. The term 'physiologic race' may be used to designate one, or a collection of entities that behave more or less consistently in parasitism on certain selected host cultivars, which are used as differentials. Geshelle (1936) suggested that each individual pure culture is a clone, the vegetative progeny of a single spore, and genetically homogeneous, and that the race is a collection of clones that may differ genetically, but react alike within the limits of differentiation selected by the adoption of an arbitrary group of differential cultivars and arbitrary categories of reaction types.

It is evident from the data that the race population is not constant, and is likely to change considerably from season to season. Of the five races isolated from the twelve collections made during 1949 and 1950, three were recovered in the collections made in the following three seasons, together with eight races not identified in the first set of collections. Similar observations were made in Germany by Frenzel (1930), who observed no apparent regularity in the geographic distribution of the thirty-three physiologic forms identified by him. Only two races out of a total of twenty isolated from the 1928 collections were again recovered in the following season. However, too few collections were made by the present writer to indicate either the prevalence, or any pattern of regional distribution, of physiologic races of crown rust in this country.

The origin and appearance of new specialized forms has always attracted attention, and the sudden occurrence in 1952 of the two races, 274 and 275, capable of attacking varieties which had hitherto resisted infection over a period of years, is of considerable interest and importance.

P. coronata avenae possesses the sexual mechanism to facilitate the development of new races through recombination on *R. cathartica*. It is considered by Murphy (1935) that the year-to-year variation in the race populations of crown rust in North America is largely due to hybridization and segregation on the alternate host, and that the constant reappearance of certain forms was explained in part by their regular hibernation in the winter oat region. Most of the forms studied by Murphy were apparently sufficiently aggressive to allow hibernation on autumn-sown or volunteer oat plants in the winter-oat region, but only a few forms appeared to overwinter successfully.

In Britain the alternate host is not widely distributed in the areas where crown rust of oats is most prevalent, and it remains to be established whether this mode of race origin plays an important role in the year-to-year variations in the race populations of this country. In the evolution of *P. coronata avenae*, however, it is evident that the production of races in this way is important in the creation of the present diversity of pathogenic types in the fungus.

In the course of this investigation some observations were made on the incidence of the disease during the autumn and on its earliness of appearance in spring in the winter oat nurseries. The dates upon which uredosori were last observed in the field on winter-hardy plants in the autumn, and when they were first observed in the spring, for the years 1949–52, are given in Table 6.

During the autumn of 1949, autumn-sown seedlings were fairly heavily infected with crown rust and well-developed uredosori continued to be produced until the onset of colder weather in the first week of December. Seedling plants showing necrosis in late December were brought in to a heated glasshouse and produced uredospores after an incubation period of 6 days. It is evident, therefore, that the perpetuation of existing races occurs in this way, but it is also probable that races vary in their reaction to environmental conditions when overwintering and that certain forms survive where others fail. The unusually early appearance of uredospores in the winter-oat nursery in 1951 is also indicative of overwintering in the uredospore state.

Table 6

Year	First date uredosori observed in spring	Last date uredosori observed in autumn
1949	—	First week in December
1950	20 April	Second week in November
1951	23 March	First week in November
1952	15 April	First week in November

In countries like Australia, where marked changes in the races of *P. triticina* have occurred despite the absence of any observed natural infection of the alternate host, modes of origin other than through sexual hybridization must be sought to explain the appearance of new races. Mutation in the diploid mycelium has been postulated as a source of origin for new physiologic races of this rust by Johnston (1930) and Roberts (1936). The origin of new races by mutation is also known to occur in *P. graminis*. In *P. graminis tritici* mutations affecting pathogenicity have been described by Stakman, Levine & Cotter (1930), Newton & Johnson (1939) and Watson (1957*a*).

Investigations have suggested that new biotypes may also arise by means of vegetative fusion of dikaryotic hyphae and subsequent exchange and regrouping of nuclei (Johnson, 1953; Waterhouse, 1932). Recently Nelson, Wilcoxson & Christenson (1955), and Watson (1957*b*) presented evidence that new races of *P. graminis* arise by this means. Nelson *et al.* reported the production of heterokaryotic biotypes which were more virulent than the biotypes of the parental races originally mixed together for the purposes of the study. These heterokaryons prove unstable but the new and temporary associations of nuclei had provided a means apart from the sexual process whereby random assortment of nuclei could occur when reverting to the dikaryophase condition.

There is no evidence that heritable changes may result in rust races as a consequence of long exposure to abnormal environments. The 'bridging host' hypothesis, whereby a race of rust that can infect one host cultivar, but not a second, may gain the ability to infect the second if it is first passed through an intermediate cultivar, has not been tested with reference to *P. coronata avenae*. In studying the effects of passage through the alternate host on the pathogenicity of various varieties of *P. coronata*, Brown (1938) found that their pathogenic characteristics were not altered appreciably by their passage through the aecidial host.

While there is no proof that 'bridging hosts' or ecological adaptation play any part in the origin of races, such factors evidently do so in changes in the composition of race populations. It is suggested by Chester (1946) that it was the latter effect which Marshall Ward mistook for fundamental changes in the pathogenicity of races. With varying environmental conditions, and with constant development and introduction of new, and elimination of old, host cultivars, it is evident that the stability of the physiologic race flora is being continuously disturbed.

The appearance of the two new races capable of attacking Bond and Victoria, which hitherto had proved resistant, may be considered as an example of host influence on the composition of the race population; both races were isolated in the breeding nurseries where large numbers of segregate lines, derived from crosses with one or other of these two cultivars as parents, were being studied. The two races may have been present previously, but in such low frequency that they were not detected in the limited surveys made. Increases in the number of types grown in the field nursery capable of resisting infection reduced competition in favour of these two races capable of attacking them; the two races were consequently given a highly selective advantage in propagation.

The data presented here serve to emphasize that, in developing crown rust resistant varieties of oats, it is important to determine the biologic forms in which the pathogen exists in a region. The economic importance of physiologic specialization lies in the fact that the existence of a multiplicity of physiologic races confers on the pathogen a versatility whereby it can attack a wide range of host cultivars, even if each individual race possesses only a narrow host range.

It is not always possible for the plant breeder concerned with the production of resistant cultivars to develop agronomically satisfactory cultivars that are resistant to all the prevailing physiologic races. If there still exist physiologic forms capable of attacking the newly introduced cultivars, however, the danger remains that these races, though perhaps previously unimportant, may assume greater prevalence and importance. The more intensively and extensively these host cultivars are grown, the greater is the danger that they may exert on the existing population of races a selective influence favouring the increase of those races to which they are susceptible. It is the selective influence of the host cultivar that determines which race, or group of races, shall prevail, and consequently the pathogenicity of new races is pre-determined by the host genotype.

The possibility of a new race increasing rapidly from an insignificant beginning to predominance is well illustrated by the spread of race 56 in wheat stem rust (*P. graminis tritici*), which was associated with the widespread cultivation of the wheat cultivar Ceres, susceptible to race 56, but resistant to many others (Stakman & Cassell, 1938). In fact, Stevens & Scott (1950), in an analysis of the acreages sown to cereal cultivars in the Central Corn Belt of the United States, were forced to conclude that new cultivars would be needed every four or five years because of the changing status of the various rust pathogens.

It is clearly apparent, therefore, that it is necessary for a new cultivar to have immunity to all races to which it is likely to be exposed if lasting resistance is to be achieved. Even if it has been possible to accomplish this, there is still no guarantee that new physiologic races may not arise, particularly in regions where the alternate host enables crossing and selfing of existing races to take place. The appearance within recent years of race 15B of *P. graminis tritici*, a biotype of race 15 capable of attacking wheat cultivars such as Thatcher and Renown, is an example of the emergence of a previously unknown strain of wheat stem rust (Hart, 1944).

It seems likely that a study of the inheritance of pathogenicity in hybrids between races should yield information on potential pathogenicity and facilitate the selection of parental material possessing resistance to any new races expected by re-combination on the alternate host. The mode of inheritance of pathogenicity in *P. coronata* has not been studied. In the case of *Melampsora lini*, Flor (1946), who studied F_2 cultures of hybrids between races, found that for each pair of genes conditioning rust reaction in the host there is a corresponding pathogenic pair in the rust fungus. When the whole concept of physiological specialization is considered from the point of view of the pathogen, as well as from that of the host, it may be well worth while considering 'moderate' resistance as opposed to 'high' resistance, which would be less highly selective on the rust population, and thereby avoid the rapid propagation of races, capable of nullifying the breeder's previous efforts in combating the disease. This would involve a further search for genes governing this type of resistance. Some progress along these lines has been already reported by Poehlman & Kingsolver (1950), who isolated lines breeding true for an intermediate type of reaction to various crown rust races from the cross of Columbia × Victoria-Richland.

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BRITISH RECORDS, 25–28

25. **Scutellinia stenosperma** Le Gal (*Les Discomycètes de Madagascar*, pp. 146–151, 1953, cum icones). Seven collections from widely separated localities establish this as a frequent but overlooked species in Great Britain. Doubtless it has often been listed as *S. scutellata* but, even in the field, the two can be known apart if the clothing of hairs is carefully observed under a lens. In *S. stenosperma* the hairs are densely serried, rigid and all of much the same length which rarely reaches 600 μ, whereas in *S. scutellata* they are more spaced apart and very unequal in length, the longest usually exceeding 1000 μ. Microscopically, hair-septation and spore shape and ornamentation provide adequate means of differentiation. Fresh material has been seen from the following localities: Dunkeld, Perth, Sept. 1953, on sawdust (No. 777); Glen Roy, Inverness, Aug. 1954, on sawdust (No. 942); Dublin, Oct. 1954, on sawdust, leg. F. G. Hassell (No. 981); Pateley Bridge, Yorks, Sept. 1955, on sodden trunk (No. 1057); Leeds, Oct. 1956, on vegetable debris, leg. La Touche (No. 1134); Hebden Bridge, Yorks, Oct. 1956, on vegetable debris, leg. R. Watling (No. 1136); Clapham, Yorks, Sept. 1957, on rotten wood (No. 1222). The numbers refer to my own herbarium. I am grateful to Mme. Le Gal for kindly confirming No. 1057 and for graciously allowing me to examine part of the type collection from Brittany. The fungus described as *Lachnae lusatiae* by Svercek (*Bohemian Species of Pezizaceae*, p. 57, 1948) is most probably *S. stenosperma*.

W. D. GRADDON, *Congleton, Cheshire*

26. **Durella suecica** (Starb.) Nannf. (*Mollisia suecica* Starb. in Sacc., *Syll. Fung.* **3**, p. 331). In crowded colonies on either surface of the cone-scales of *Pinus sylvestris*, near Forres, Moray, May 1957, leg. I. D. Finney. Herb. W.D.G. 1183 and Herb. Kew. The smooth, black apothecia are shallow saucer-shaped and up to 0·6 mm. across. The highly characteristic excipulum consists of stout, thick-walled, dark brown hyphae in parallel rows which leave the hypothecium more or less tangentially and then curve upward slightly to join the cortical surface; these hyphae have several slender, hyaline septa. There is a minute hyaline footstalk resting just within the host tissue. Asci 50 × 6 μ, the pore blue in Melzer reagent; spores fusiform with rather acute ends, 6·5–8·5 × 2 μ. Clearly the species is closely akin in structure to *D. commutata* Fuckel of deciduous wood (see Dennis in *Mycol. Pap. C.M.I.* **62**, 14, 1956).

W. D. GRADDON, *Congleton, Cheshire*

27. **Pezicula myrtillina** Karst. (*Mycol. fenn.* **1**, 165) (Syn: *Orbilia boydii* A. L. Sm. & Ramsb., fide Nannfeldt in *Trans. Brit. mycol. Soc.* **20**, p. 199, 1936). In quantity scattered along a dead, dry, aerial branch of *Vaccinium myrtillus* near Dane Cottage, N. Staffs., July 1957. Herb. W.D.G. 1194. Small greyish white domes to 0·6 mm. across, densely pulverulent. Spores

22–28 × 5·5–6 μ, fusiform, either filled with small guttules except for a clear median belt or with two large guttules and a few small ones. Paraphyses with a slender, septate filament carrying a large globose or pyriform head to 12 μ across; these heads overtop the asci by as much as 30 μ and give to the hymenial surface its powdery appearance. Apothecia erumpent from an extensive, deep-seated, small-celled, hyaline stroma. Excipulum of colourless textura prismatica of more or less isodiametric cells on the flank but of narrow elongate cells ranged in parallel series at a low angle to the surface, in the perihymenial region; there is no sharp boundary between the excipulum and the inner fleshy tissue.

W. D. GRADDON, *Congleton, Cheshire*

28. **Belonopsis graminea** (Karst.) Sacc. & Syd. (*Syll. Fung.* **16**, p. 753, 1902.) In quantity on stems of *Agropyron* sp. near Pickering, Yorks, August 1957, leg. W. G. Bramley. Herb. W.D.G. 1211. Karsten's type material was studied and described by R. W. G. Dennis (*Kew Bull.* 1950, p. 182) and this collection matches exactly. Spores 24–26(–30) × 3 μ, fusoid with acute ends and brilliantly 3-septate. This is a species with a very distinct aspect in the field; the apothecia are rather scattered and are individually ringed with a zone of dark radiating hyphae on the host; the pallid disc contrasts strongly with this zone and with the dark cortex; they are easily detached and a pale spot ringed by the zone of dark hyphae is left exposed by their removal. In 1950 I collected this species on *Elymus riparius* at Albany, N.Y., U.S.A. Material under this name in Herb. Hull is typical *Mollisia mutabilis* (B. & Br.) Mass. on *Deschampsia caespitosa*.

W. D. GRADDON, *Congleton, Cheshire*

THE DUBLIN FORAY

13 TO 19 SEPTEMBER 1957

The Autumn Foray was held in Dublin, Eire, from 13 to 19 September, and was attended by about thirty-four members. Accommodation and a work-room was provided at Trinity Hall by kind permission of the authorities of Trinity College, Dublin.

The first excursion, on Friday afternoon, was to Powerscourt Estate, Co. Wicklow. On Saturday 14 September we visited the State Forest at Woodenbridge and Sheldon, Co. Wicklow, where after a day's collecting we were given a most welcome and generous tea at the Forestry School. Townley Hall, Co. Louth, was visited on Monday 16 September. Here we searched in drenching rain but were rewarded in the afternoon by finding a beautiful stand of *Spathularia flavida* and a collection of *Cordyceps ophioglossoides*. Later in the afternoon we drove to see the Newgrange tumulus, and most members squeezed and sidled their way to the interior. The second half-day Tuesday 17 September, was spent at the Carton Estate, Co. Kildare, and the final day, Wednesday 18 September, at Glendalough. This is the excursion that will be remembered longest, the weather was warm and sunny and we spent a delightful day.

No comments were sent in with the lists of fungi. Conditions were rather poor for collecting as the summer months had been dry in Eire in contrast to the almost continual rain we suffered in this country. The main reason, however, for the briefness of the agaric list was the absence of our experts in this group. I am most grateful to the members who threw in all the names they knew in an attempt to list the agarics, but we were all shamed by the speed with which the large dustbin, kept under the display table, filled with 'unknowns'!

We were entertained on Monday evening by the authorities of University College, Dublin, when we had the pleasure of meeting the President, Dr Tierney; the Head of the Botany Department, Prof. Doyle, and several members of his staff. After the reception, a business meeting was held in the Botany Lecture Theatre by kind permission of Prof. Doyle. After thanking the organizers of the Foray and all who had contributed to make it such a success, the President, Dr Hickman, announced that the 1958 Foray will be a joint meeting with the Société Mycologique de France and, at the request of our guests, will be held in Reading.

We spent a very pleasant Sunday morning with Dr O'Connor who very kindly invited all members to take coffee with him at his home.

Dr A. H. S. Brown, Dr L. E. Hawker, and Messrs Colhoun, O'Connor, Hickman, Ingold, Manners, Palmer, and Webster have all contributed to the List of Species, and many members helped to sort and arrange the collections on the work-room tables. I am grateful to them all. The arrangements and organization of this Foray were carried out by Dr Stuart and Dr O'Connor, and to their hard work, which started as far back as October 1956, the success of this Foray is due.

List of Species

B. = Bull Island, Co. Dublin; *C.* = Carton Estate, Co. Kildare; *D.* = Dargle Glen, nr. Bray, Co. Wicklow; *G.* = Glendalough, Co. Wicklow; *K.* = Killakee Mt., Co. Dublin; *P.* = Powerscourt Estate, Co. Wicklow; *S.* = Sheldon Forest, Co. Wicklow; *T.* = Townley Hall, Co. Louth.

Nomenclature follows the British Lists [see these *Transactions*, **38**, 185, 1955], where possible. Author citations are given only when the fungal name used does not appear in the appropriate British list.

PHYCOMYCETES

Albugo candida, on *Capsella bursa-pastoris*, *P.*
Peronospora parasitica, on *Capsella bursa-pastoris*, *P.*

HEMIASCOMYCETES

Taphrina tosquinetii, on *Alnus*, *P.*

EUASCOMYCETES

Pyrenomycetes

Chaetosphaeria callimorpha, on *Rubus*, *S.*; myriocarpa (Fr.) Booth, on *Fagus*, *G.*, *P.*, *T.*
Cordyceps capitata, on *Elaphomyces granulatus*, *G.*; militaris, *G.*; ophioglossoides, on *E. muricatus*, *G.*, *T.*
Dialonectria peziza, on *Fagus*, *G.*
Diaporthe arctii, on *Arctium*, *P.*, *T.*
Diaporthopsis angelicae, on *Angelica*, *P.*
Didymosphaeria brunneola Niessl, on *Hypericum*, *S.*
Epichloe typhina, on *Dactylis glomerata*, *P.*
Erysiphe umbelliferarum de Bary, on *Heracleum spondylium*, *G.*
Gloniopsis levantica Rehm, on *Quercus*, *W.*, *Rubus fruticosus*, *S.*
Hypoxylon fuscum, on *Corylus*, *S.*; fragiforme (Fr.) Kickx., *T.*; serpens, on *Fagus*, *G.*, *P.*
Leptosphaeria clivensis, on *Senecio jacobaea*, *S.*; rusci, on *Ruscus*, *C.*
Lophiosphaera fuckelii, on *Rubus*, *C.*
Lophiotrema vagabundum, on *Geum urbanum*, *T.*, on *Rumex*, *P.*
Melanomma pulvis-pyrius, *S.*; subdispersum (Karst.) Berl. & Vogl. apud Sacc., on *Fagus*, *G.*
Melomastia mastoidea, on *Hypericum androsaemum* *S.*
Microsphaera alphitoides Griff. & Maubl., on *Quercus*, *G.*
Nectria cinnabarina, *P.*, *T.*
Ophiobolus rubellus, on *Senecio jacobaea*, *S.*
Phomatospora berkeleyi, on *Urtica*, *P.*
Pleospora herbarum, on *Epilobium montanum*, *T.*, *Galium aparine*, *T.*
Stigmatea robertiani, on *Geranium robertianum*, *T.*
Uncinula aceris, on *Acer pseudoplatanus*, *G.*
Ustulina deusta (Fr.) Petrak, *S.*
Xylaria carpophila, on *Fagus*, *G.*; hypoxylon, *G.*; polymorpha, *S*, *T.*

Discomycetes

Chlorociboria aeruginosa (Fr.) Seaver, *S.*
Coryne sarcoides, *P.*
Dasyscypha virginea, *T.*
Galactinia succosa, *S.*
Helvella crispa, *T.*; lacunosa, *T.*
Leotia lubrica, *G.*, *S.*
Microglossum viride, *S.*
Otidea leporina, *T.*

Peziza aurantia, *G.*, *P.*, *T.*
Phaeobulgaria inquinans (Fr.) Nannf., *G.*, *T.*
Pseudopeziza ranunculi, on *Ranunculus repens*, *G.*
Rhizina inflata, *G.*
Rhytisma acerinum, on *Acer pseudoplatanus*, *P.*
Sclerotinia curreyana, on *Juncus conglomeratus*, *G.*
Scutellinia scutellata, *G.*
Spathularia flavida, *T.*
Stegia ilicis, *T.*
Trichoscyphella hahniana, on *Larix decidua*, *S.*; willkommii on *Larix decidua*, *G.*

HETEROBASIDIOMYCETES

Uredinales

Coleosporium senecionis, on *Senecio vulgaris*, *C.*, *S.*, *T.*; sonchi, on *Sonchus asper*, *P.*, on *S. oleraceus*, *T.*; tussilaginis, on *Tussilago farfara*, *T.*
Melampsora hypericorum, on *Hypericum androsaemum*, *S.*
Melampsoridium betulinum, on *Betula verrucosa*, *G.*, *S.*
Milesia blechni, on *Blechnum spicant*, *G.*, *S.*, *T.*; kriegeriana, on *Dryopteris austriaca*, *S.*, on *D. spinulosa*, *G.*; scolopendrii, on *Phyllitis scolopendrium*, *C.*, *P.*
Phragmidium mucronatum, on *Rosa* sp., *P.*; rubi-idaei, on *Rubus idaeus*, *G.*; violaceum, on *Rubus caesius*, *P.*, on *R. fruticosus*, *C.*, *G.*, *P.*, *S.*, *T.*
Puccinia annularis, on *Teucrium scorodonia*, *G.*, *S.*; arenariae, on *Melandrium rubrum*, *P.*; arrhenatheri, on *Arrhenatherum elatius*, *P.*; brachypodii, on *Brachypodium sylvaticum*, *P.*, *S.*; bromina, on *Zerna ramosa*, *T.*; buxi, on *Buxus sempervirens*, *C.*, *G.*; caricina, on *Carex pendula*, *G.*, *P.*, *T.*; circaeae, on *Circaea lutetiana*, *C.*, *G.*, *P.*, *S.*, *T.*; cirsii, on *Cirsium palustre*, *S.*; cnici, on *Cirsium vulgare*, *P.*, *T.*; coronata, on *Arrhenatherum elatius*, *T.*, on *Avena* sp., *T.*, on *Holcus lanatus*, *S.*, on *H. mollis*, *P.*; glechomatis, on *Glechoma hederacea*, *C.*, *P.*; glumarum, on *Dactylis glomerata*, *C.*, *P.*; graminis on *Dactylis glomerata*, *C.*; obscura, on *Luzula sylvatica*, *G.*, *S.*; poarum, on *Tussilago farfara*, *C.*; pulverulenta, on *Epilobium montanum*, *P.*, *T.*; saniculae, on *Sanicula europaea*, *G.*, *P.*; taraxaci, on *Taraxacum officinale*, *P.*
Pucciniastrum circaeae, on *Circaea lutetiana*, *C.*, *G.*, *P.*, *S.*, *T.*; epilobii on *Chamaenerion angustifolium*, *T.*
Thekopsora vacciniorum, on *Vaccinium myrtillus*, *G.*
Triphragmium ulmariae, on *Filipendula ulmaria*, *C.*
Uromyces fabae, on *Vicia sepium*, *P.*; scrophulariae, on *Scrophularia aquatica*, *C.*

Ustilaginales

Urocystis anemones, on *Ranunculus repens*, *C.*, *P.*
Ustilago longissima, on *Glyceria maxima*, *C.*

HOMOBASIDIOMYCETES—Hymenomycetes

Aphyllophorales

Clavaria corniculata, *C.*; cristata, *P.*; kunzei, *P.*
Daedalea biennis, *P.*
Fomes annosus, *P.*
Ganoderma applanatum, *C.*, *P.*
Hydnum repandum, *G.*, *S.*
Polyporus adustus, *P.*; betulinus, *G.*, *P.*, *S.*; giganteus, *C.*, *P.*
Polystictus abietinus, *G.*; versicolor, *G.*, *P.*, *T.*
Sparassis crispa, *P.*
Stereum hirsutum., *S*, *T.*
Thelephora terrestris, *G.*
Trametes gibbosa, *P.*, *T.*

Agaricales

Amanita citrina, *G.*, *S.*; muscaria, *G.*, *P.*; pantherina, *G.*, *S.*; phalloides, *P.*; rubescens, *S.*; spissa, *G.*, *S.*
Amanitopsis fulva, *G.*; vaginata, *S.*
Anellaria separata, *G.*
Armillaria mellea, *C.*, *G.*; mucida, *C.*, *P.*
Boletus bovinus, *G.*; chrysenteron, *P.*; edulis, *S.*; luteus, *G.*; parasiticus, on *Scleroderma aurantium*, *S.*; scaber, *G.*, *S.*; subtomentosus, *G.*, *S.*; versipellis, *G.*
Cantharellus cibarius, *G.*, *P.*; tubaeformis, *G.*
Clitocybe aurantiaca, *P.*; infundibuliformis, *C.*, *T.*
Clitopilus prunulus, *P.*
Collybia distorta, *T.*; maculata, *S.*; platyphylla, *S.*; radicata, *P.*, *T.*
Coprinus comatus, *C.*, *G.*; micaceus, *G.*, *P.*, *S.*, *T.*; niveus, *G.*; plicatilis, *C.*
Cortinarius anomalus, *G.*, *S.*; cinnamomeus, *S.*; collinitus, *G.*; elatior, *G.*, *T.*; torvus, *S.*
Craterellus cornucopiodes, *P.*
Crepidotus mollis, *C.*, *S.*, *T.*
Entoloma sericeum, *G.*
Flammula sapinea, *G.*, *S.*
Galera hypnorum, *G.*
Gomphidius glutinosus, *C.*
Hebeloma mesophaeum, *C.*
Hygrophorus calyptraeformis, *G.*; chlorophanus, *G.*; conicus, *C.*, *P.*; eburneus, *S.*; hypothejus, *G.*; miniatus, *G.*; nigrescens, *G.*; obrusseus, *G.*; pratensis, *G.*; psittacinus, *S.*; unguinosus, *G.*; virgineus, *C.*, *S.*
Hypholoma fasciculare, *G.*, *S.*; hydrophilum, *G.*; sublateritium, *S.*
Inocybe geophylla var. lilacina, *S.*; pyriodora, *C.*
Laccaria amythestina, *G.*; laccata, *G.*, *P.*, *S.*
Lactarius blennius, *G.*, *T.*; chrysorheus, *G.*; deliciosus, *G.*, *S.*; glyciosmus, *G.*; plumbeus, *S.*; piperatus, *G.*; quietus, *S.*; rufus, *G.*, *T.*; subdulcis, *G.*; torminosus, *T.*; vellereus, *G.*
Lepiota amianthina, *G.*; cristata, *C.*, *P.*; procera, *P.*, *S.*; rhacodes, *C.*
Leptonia lampropus, *C.*; serrulata, *P.*
Marasmius acervatus, *T.*; androsaceus, *P.*; confluens, *P.*, *T.*; dryophilus, *G.*, *T.*; peronatus, *G.*, *T.*; rotula, *P.*, *T.*
Mycena adonis, *S.*; galopus var. nigra, *G.*; pura, *P.*, *T.*; sanguinolenta, *G.*
Nolanea staurospora, *C.*
Omphalia fibula, *G.*, *S.*
Panus stipticus, *P.*; torulosus, *S.*
Panaeolus campanulatus, *G.*; sphinctinus, *G.*
Paxillus involutus, *G.*
Pholiota aurivella, *S.*; spectabilis, *C.*, *T.*; squarrosa, *P.*
Pluteus cervinus, *G.*, *S.*; phlebophorus, *C.*
Psathyra obtussata, *T.*
Psathyrella disseminata, *P.*
Psalliota arvensis, *P.*
Pleurotus petaloides, *T.*
Russula adusta, *G.*; atropurpurea, *G.*, *P.*, *S.*, *T.*; caerulea, *G.*; cyanoxantha, *P.*, *T.*; delica, *S.*; emetica, *G.*, *T.*; fellea, *S.*, *T.*; grisea, *G.*; mairei, *P.*; nigricans, *G.*, *S.*, *T.*; ochraleuca, *G.*, *P.*, *T.*; velenovskyi, *G.*; vesca, *S.*; xerampelina, *G.*
Stropharia aeruginosa, *G.*; semiglobata, *G.*, *S.*
Tricholoma fulvum, *G.*; ionides, *T.*; nudum, *C.*, *P.*; rutilans, *G.*, *P.*; sulphureum, *T.*; terreum, *G.*, *P.*, *T.*

HOMOBASIDIOMYCETES—Gasteromycetes

Bovista plumbea, *B.*
Lycoperdon candidum, *G.*; depressum, *B.*, *C.*; molle, *C.*, *P.*, *S.*; perlatum, *P.*, *T.*; perlatum var. nigrescens, *G.*; pyriforme, *C.*, *P.*, *S.*; spadiceum, *B.*, *K.*
Mutinus caninus, *C.*, *P.*, *S.*, *T.*

Nidularia denudata, *G.*, *K.*; minutissima J. T. Palmer, *G.*, *K.*
Phallus impudicus, *C.*, *S.*
Scleroderma aurantium, *S.*, *L.*, *T.*; bovista, *C.*, *P.*, *T.*; verrucosum, *C.*, *P.*, *S.*, *T.*
Sphaerobolus stellatus, *G.*

FUNGI HYPOGEI

Phycomycetes

Endogone macrocarpa. *T.*; microcarpa, *P.*

Ascomycetes

Elaphomyces granulatus, *G.*; muricatus, *G.*, *T.*
Tuber dryophilum, *C.*; puberulum, *P.*, *T.*; rapaeodorum, *T.*; rufum, *P.*

BASIDIOMYCETES

Hymenogaster luteus, *P.*; tener, *S.*, *T.*; vulgaris, *T.*

FUNGI IMPERFECTI

Coelomycetes

Coniothyrium equiseti, on *Equisetum*, *P.*
Macrophoma cylindrospora on *Hedera*, *S.*
Marssonina juglandis, on *Juglans*, *T.*
Pestalotia funerea, *Araucaria imbricata*, *S.*
Phoma endorhodia, on *Lapsana communis*, *S.*
Septoria polygonorum, on *Polygonum persicaria*, *G.*

Hyphomycetes (non-aquatic)

Brachysporium britannicum Hughes, on *Fagus*, *P.*; bloxami (Cooke) Sacc., on *Betula*, *P.*
Graphium calicioides (Fr.) Cooke & Massee, on *Fagus*, *G.*, *T.*
Heterosporium magnusianum Jacques, on *Narthecium ossifragum*, *G.*
Menispora ciliata Corda, on *Betula*, *G.*, *Fagus*, *P.*, *T.*
Monotospora sphaerocephala Berk., on *Fagus*, *T.*
Ovularia obliqua (Cooke) Oud., on *Rumex obtusifolius*, *C.*, *G.*; veronicae (Fckl.) Sacc. on *Veronica chamaedrys*, *G.*
Paecilomyces elegans (Corda) Mason & Hughes, on *Fagus*, *T.*, *Pinus*, *G.*
Passalora depressa (B. & Br.) Höhn., on *Angelica sylvestris*, *G.*
Polythrincium trifolii Kunze & Schm., on *Trifolium repens*, *T.*
Pycnostysanus azaleae (Peck) Mason, on *Rhododendron*, *G.*
Periconia byssoides Pers. ex Schweinitz, on *Rubus*, *P.*
Ramularia sambucina Sacc., on *Sambucus nigra*, *C.*; scrophulariae Fautr. & Roum. on *Scrophularia nodosa*, *G.*; urticae Ces., on *Urtica dioica*, *C.*, *G.*; variabilis Fckl., on *Digitalis purpurea*, *G.*
Tripospermum myrti (Lind) Hughes, *C.*

Hyphomycetes (aquatic)

Alatospora acuminata Ingold, *D.*, *G.*, *T.*
Anguillospora longissima (de Wild.) Ingold, *D.*, *G.*, *T.*
Articulospora tetracladia Ingold, *D.*, *T.*
Clavariopsis aquatica de Wild., *D.*, *G.*, *T.*
Dactylella aquatica (Ingold) Ranzoni, *D.*, *T.*
Dendrospora erecta Ingold, *G.*
Flagellospora curvula Ingold, *D.*, *G.*, *T.*
Heliscus aquaticus Ingold, *D.*, *G.*, *T.*; longibrachiatus Ingold, *D.*; stellatus Ingold & Cox, *D.*
Lemonniera aquatica de Wild., *D.*, *T.*
Lunulospora curvula Ingold, *D.*, *G.*, *T.*
Margaritispora aquatica Ingold, *D.*, *G.*
Tetrachaetum elegans Ingold, *D.*
Tetracladium marchalianum de Wild., *C.*, *D.*, *T.*; setigerum (Grove) Ingold, *T.*
Tricladium angulatum Ingold, *C.*; gracile Ingold, *D.*, *G.*; splendens Ingold, *G.*, *T.*

PROCEEDINGS

MEETINGS

Meeting held at Birkbeck College, University of London, at 11.0 a.m., 11 January 1958. The President, Dr F. B. Hora, in the Chair.

G. A. Salt. *Oospora pustulans* as a parasite of potato roots.

The fungus *Oospora pustulans* Owen & Wakef., which causes the skin-spot disease of potato tubers, also causes a brown rot of the underground part of the stems, roots and stolons of potato plants. The symptoms on roots, first noticed at Rothamsted in 1951, occur early in the season and are usually most severe at the stem base where it joins the seed tuber. Rotting is confined to the outer cortex which splits deeply along its axis, and less deeply at right angles to it. There is no evidence yet that root infection affects plant growth or yield, but a decrease in tuber number and a corresponding increase in their individual size has been indicated.

At Rothamsted *O. pustulans* was prevalent on roots of all potato crops examined in August between 1953 and 1957, even where potatoes had not been grown previously. The fungus was also on the roots of all N.I.A.B. maincrop varieties at six centres visited each year from 1955 to 1957. Its incidence varied with soil type and cultivar, it was consistently less on Black Fen than on other soil, and less on cultivars Ulster Supreme and Arran Viking than on Majestic and King Edward.

Cultivar differences in the incidence of root infection were directly related to the proportion of infected eyes on the seed, and not to the surface area of tuber covered with pustules. Eyes excised from samples of seed tubers and incubated at 16° C. for 5 days, showed that some cultivars with no visible pustules, e.g. Ulster Torch, had many infected eyes and grew extensively browned roots. Eye tests on four random samples of Majestic seed showed 65–80 % tubers infected, whereas only 0–30 % had visible lesions and would normally have been recorded. The fungus may not therefore be such a common soil inhabitant as generally believed; investigations into its existence in soil are facilitated by the discovery that the fungus attacks tomato roots, causing brown lesions. Tomato seedlings are grown in pots of field soil for 8–10 weeks and the fungus identified on root lesions by sporulation.

I. F. Storey. The source and control of Apple Scab infection at Wisbech.

For many years the control of Apple Scab has presented problems in the apple-growing area centred on Wisbech. Bramley's Seedling, a susceptible cultivar, is widely grown and the low lying fertile soils facilitate the development of the disease. In some seasons ten or more fungicidal sprays would be applied without affecting any real measure of control.

Our first year's observations showed that the leaves and fruit were becoming infected at an early stage in their development, and we attempted to find the source from which these early infections arose. Using a Hirst spore trap in 1953 in an orchard, it was clearly seen that the ascospores were responsible for these early infections—although traces of bad scale infection such as Dr McKay observed in Ireland had been found in 1952.

During the time these observations were being made a change-over was taking place in the type of fungicide used for scab control—mercurial sprays were largely replacing materials such as lime sulphur and Bordeaux mixture. From surveys made in the orchard it became apparent that the mercurials were more effective because they had better fungistatic properties than the other materials. In addition, spraying immediately after wet spells produced exceptionally good results. Our results were in agreement with those of workers in other countries and confirmed that the mercurial fungicide was largely curative in action.

In order to apply the fungicide to the best advantage it is essential to know when infection is taking place, and to obtain the best results the sprays must be applied within 4 days of infection.

The data which have been widely used for this purpose are those of W. D. Mills who tabulated the period of leaf wetness required for infection to take place at varying temperatures. At the outset problems arose as to the proper measurement of temperature, and the determination of leaf wetness. The severity categories he proposes only really have a meaning in relation to the spore numbers causing infection.

The measurement of leaf wetness was determined by several means—visually and by means of the dew balance. Later the surface wetness recorded was developed for this purpose. Difficulties also arise in relation to the interpretation of the data, e.g. how important is dew wetting, and the permissible length of dry breaks.

The curative method of fungicide application was tested on a wider scale for the first time in 1955, when the first infections took place on 27 April following a long dry spell. The effect of accurate timing of the sprays in relation to this date was apparent, both on the leaves and also on the fruit right up to picking time.

This same trend has been observed in subsequent years, and this method of application has led to a reduction in the number of sprays required and also to a very high level of control.

The results show that in many seasons there are only a few occasions during the period of ascospore release when infection takes place, and it is important to effect a control at these times. Looking back on the literature, most of the seasons when scab control has been difficult have been those when scab has appeared at an early date (e.g. end of April) and heavy infection had probably taken place before the first fungicides had been applied to the tree. We have seen from spore trapping how heavy the spore load in the atmosphere can be during the latter half of March.

J. M. HIRST. Quantitative studies of Apple Scab attacks.

Aerobiological studies to define the type of spore causing the first apple scab infections each year pointed to the predominant importance of ascospores at Wisbech (Hirst *et al.* 1955). Later work has therefore aimed to improve fungicidal control. During periods of rapid growth it is difficult to protect foliage completely and continuously because it is only the young parts of apple trees which are highly susceptible. The development of fungicides which can kill established infections offered a new possibility for controlling the disease, provided they could be timed accurately within the brief period after infection during which they are lethal.

Dramatic reductions in the number of ascospores caught in two Wisbech orchards have followed the introduction of phenyl mercury sprays timed for curative action. Times of infection were indicated, according to the infection requirements proposed by Mills (Mills & LaPlante, 1951), from the records of thermographs and surface-wetness recorders (Hirst, 1957). The reduced number of ascospores cannot yet be attributed to these treatments because it has not been possible to assess reductions due to natural causes in the sprayed orchards where the observations had to be made.

To overcome some of these difficulties, small wind tunnels have been developed for estimating the relative annual ascospore productivity per unit area of dead apple leaf from different orchards or plots. Large differences in productivity have been found from season to season, with different orchard managements or following the application of DNOC to dead leaves in spring.

There have been great discrepancies between the relative order of ascospore productivity per unit area of dead leaf from different orchards and of the concentrations of ascospores measured by spore traps in the orchards. It seems that these are caused largely by differences in the extent of the source, e.g. weight of dead leaf per unit area of ground, resulting from variable persistence of dead leaf from autumn to spring. The extent of such differences and their cause is at present being studied.

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MILLS, W. D. & LAPLANTE, A. A. (1951). Diseases and insects in the orchard. *Cornell Ext. Bull.* no. 711, 21–27.

P. A. Dixon. The life cycle of *Ascocybe grovesii*

C. T. Ingold. Physiological study of spore discharge in *Sordaria.*

Sordaria fimicola grows readily on filter-paper yeast-extract agar producing a fruiting culture discharging spores in 8–10 days at 20° C. Cultures grown in continuous white light (*c.* 100 f.c.) or in complete darkness fruit equally well and from both spore discharge occurs. A culture raised in alternating light and dark (12 hr. L, 12 hr. D/day) shows a periodicity of spore liberation, when discharge starts, with a low level in the dark periods and a steep increase in the light periods to a maximum followed by a fall (but not to the level of the rate in the dark). On keeping subsequently in continuous darkness the low dark level is maintained and there is no sign of an endogenous rhythm such as occurs in *Daldinia.* The magnitude of the peak in discharge rate in a period of illumination following one of darkness is related to the length of the dark period.

Using light of equal intensity (based on thermopile measurement) but of a different wave-length, it is found that light-stimulated spore discharge is sensitive to radiation between 450 and 500 mμ, but not to that between 500 and 700 mμ. A rough action-spectrum of light-stimulated discharge shows a rough correspondence with the absorption spectrum of a yellow-orange pigment extractable from the fungus with ethyl alcohol.

The behaviour of a melanin-free mutant (with yellow-orange perithecia and colourless spores) has been compared with the normal. As in the normal, spore discharge increases rapidly on transfer from dark to light; however, response is faster in the mutant suggesting that in the normal the melanic pigment in the outer layers of the perithecial wall acts as a neutral filter reducing the effectiveness of the incident light. In this connexion it is to be noted that in the normal form response to lower light intensities is slower than at higher intensities.

Two other light-sensitive processes in *Sordaria,* phototropism of the perithecial necks and the production of the yellow-orange pigment, have been shown to have action spectra roughly corresponding to that of light stimulated-spore discharge. It is suggested that light may affect some basic photochemical step involved in all three processes.

P. H. Gregory and E. J. Guthrie. Elementary experiments on spore dispersal by splash droplets.

Meeting held at the London School of Hygiene and Tropical Medicine at 11.0 a.m., 8 March 1958. The President, Dr F. B. Hora, in the Chair.

SOME MECHANISMS OF VARIATION IN FUNGI

G. Pontecorvo. The parasexual cycle.

J. L. Jinks. Cytoplasmic variation in fungi.

The fungi, especially those which readily form heterokaryons and whose sexual cycle allows some degree of outbreeding, provide some of the most favourable material for investigating the role of the cytoplasm in heredity and development. As a consequence there has been a steady accumulation of well-established examples of cytoplasmic variation in such diverse material as the yeasts, *Neurospora crassa,* the *Penicillia* and *Aspergilli*, *Podospora anserina*, *Coprinus lagopus*, *Puccinia graminis* and *Pholiota mutabilis* while a cytoplasmic basis can be inferred from the behaviour of many colonial variants described in the literature, especially amongst those which exhibit a so-called 'dual' nature.

Three types of cytoplasmic change are theoretically possible: (1) change in the equilibria of cytoplasmic elements; (2) a loss, or change of function in a cytoplasmic element; and (3) the physical loss of a cytoplasmic element: all of which have been claimed to contribute to cytoplasmic variation.

Some of the cytoplasmic changes result in quantitative differences in phenotype. Initially these are small and difficult to demonstrate, but when protected from the operation of natural selection they can be built up into large differences of major consequence to the fungus. Such changes can be accelerated or reversed by conscious selection while sexual propagations or the heterokaryotic exchange of cytoplasm with normal clones removes them. They occur most frequently in aged clones which have been propagated vegetatively, and they rarely involve permanent changes in the nature or function of cytoplasmic elements.

Other cytoplasmic changes appear from the first as major variants, either as sectors in normal colonies or as segregants in their asexual or sexual progenies. Initially the abnormal sectors or segregants contain a mixture of normal and mutant representatives of some cytoplasmic element, hence their own progenies segregate to give both normal and abnormal phenotypes, the majority of which also possess mixed cytoplasms. Mixed cytoplasms can also be obtained by making heterokaryons between two homokaryons, one or both of which carry mutant cytoplasmic elements. In most mixed cytoplasms, so far investigated, the components interact in some complex way which leads to the suppression and ultimate loss of one of the components.

Ultra-violet irradiation, acriflavine, high temperatures and subnormal media will induce cytoplasmic changes. Some treatments have a general effect producing a range of variants while others, e.g. acriflavine, are specific, producing the same variant with a high frequency whenever used.

Some treatments lead to adaptive cytoplasmic changes. These have been obtained in response to toxic substances in the medium, e.g. mercuric chloride, or to the presence of alternative carbon sources. No permanent changes in the cytoplasm are involved: only reversible adjustments in the equilibria of cytoplasmic elements.

These observations leave no doubt as to the importance of the cytoplasm in heredity and development. Other observations, however, are equally clear in showing the ultimate dependence of the cytoplasm on the nucleus; variation in the former being limited by the requirements for successful nuclear-cytoplasmic co-operation.

P. R. DAY. Mutation to virulence in plant pathogens.

In many, if not all, plant pathogens physiologic specialization is the outcome of a gene-for-gene relationship between host and parasite where each resistance gene may be countered by an equivalent gene for virulence. The few exceptions (complementary gene action for virulence to a single resistance gene in rust on Bond oats, unexplained adaptation to disomic resistant hosts in potato late blight) strengthen this view.

While heterocaryosis and sexual and parasexual recombination recombine pathogen genes new virulence alleles arise only as a result of point changes in the gene. They are recognized by their virulent phenotype when tested against a complementary resistant gene. Attempts to induce mutations of this kind have been made with maize wilt, apple scab, oat stem rust, blast disease of rice, flax rust and leaf-mould disease of tomato.

My own work on *C. fulvum* has been along the following lines:

(i) The sensitivity of the virulent mutant selection method was tested in reconstruction experiments. The method can detect about seventeen virulent conidia in 10^6 avirulent conidia.

(ii) Conidia were harvested from mycelia pre-treated with X-rays and ultra-violet so that the difficulties of: (*a*) delayed expression of mutation, (*b*) competition of wild type sister cells in a conidium with a single mutant cell, (*c*) virulent conidia numbers below the infection threshold, could be overcome.

(iii) Markers, to be used to exclude contamination, were induced and tested for pathogenicity to non-resistant hosts. Of fifty-one biochemical mutants only two were pathogenic. This parallels results with other pathogens (*Venturia*, *Erwinia*, *Klebsiella*, etc.). Twenty-one out of twenty-two pigment mutants were pathogenic.

(iv) One induced virulent mutant with a red pigment marker has been recovered so far. (At the moment I am working to repeat this result during the summer.)

The results of these experiments have implications for: (i) the nature of disease resistance—suggesting some ways of approaching the problem; (ii) the indiscriminate treatment of plants and their pathogens, which may unintentionally be present, with mutagens.

E. W. Buxton. Pathogenic variation in *Fusarium oxysporum*.

There are three known possible ways in which new pathogenic isolates may arise in fungi: mutation, re-association and recombination of already existing genetic material, and by a system similar to adaptation.

There is no definite evidence of mutation playing an important part in the very frequent changes in morphology and in pathogenicity which occur in *Fusarium*.

Heterokaryosis—the association of two different nuclei from weakly pathogenic isolates of *Fusarium oxysporum* f. *pisi* race 1 (cause of pea wilt) in one mycelium causes a great increase in pathogenicity towards pea cultivar Onward. The probable significance of heterokaryosis in nature, particularly in attempting to explain the sudden appearance of wilt disease in areas where it has never previously been known, and in explaining the protracted survival of pathogens in soil, is discussed.

Parasexualiam—diploidization occurs in heterokaryotic mycelia of imperfect fungi (Pontecorvo) and this has been demonstrated in pathogenic races of *F. oxysporum* f. *pisi*. This phenomenon results in the formation of recombinant progeny which include new pathogenic types of the fungus. Some of these new types are able to wilt pea cultivars hitherto only wilted by race 3. It appears that new races can arise by this recombination of already existing genetic differences. A similar problem is being investigated elsewhere in wheat rusts, in which new races are arising in experiments conducted in the absence of the perfect stage.

Adaptation—it has been shown in the pea wilt syndrome that exudates from roots of pea cultivars, which are used for differentiating pathogenic races of *F. oxysporum* f. *pisi*, affect spore germination in different ways. Race 1, for instance, cannot germinate well in exudate from roots of the resistant cultivar Alaska. When, however, spores of race 1 are retained in Alaska exudate for 14 days at 24° C., they gradually become able to wilt Alaska when later used as inoculum. Spores retained in sterile water or in exudate from the susceptible cultivar Onward were unable to do this. Such temporary yet clear-cut modifications brought about by this pre-treatment of pathogenic race 1 appear, therefore, to result in effects similar to those exerted by races 2 and 3. The nature of the mechanism is not fully understood, but it may be similar to the adaptive systems described in bacteria.

A re-examination of past work suggests that such 'adaptation' may frequently occur in pathogenic fungi (e.g. work by Marshall Ward, Salmon, D'Oliviera, De Vay, Peterson, Hroshovetz, etc.).

K. Mather. Concluding remarks.

REVIEWS

A Manual of Soil Fungi. By J. C. Gilman. (Ames: Iowa State College Press, 1957.) 2nd edition, pp. x+450. 153 text-figures, 13 plates. Price: $7·50.

It is pleasing to see a new edition of Dr Gilman's well-known and useful *Manual of Soil Fungi*. The new edition has been enlarged from 392 to 450 pages, with the inclusion of many new genera and species collected together from widely scattered sources. This has greatly increased the usefulness of the book, particularly with reference to genera such as *Mortierella* known otherwise only by recourse to the original papers of Linnemann. Besides the added species, others present in the original edition have, in accordance with modern work, been reduced to synonymy, though perhaps Bisby's work on *Stachybotrys* has been overlooked. Line drawings have been added for most of the newly admitted genera, but the old cuts used in the first edition, many of them not too satisfactory, have been retained. The plates prove a welcome addition to the text, but it does seem a pity to me that, while many genera are not illustrated, about one-quarter of the plates are devoted to *Aspergillus* and *Penicillium*. While these well-known genera are abundant in soil, anyone making critical determinations in either genus will have to have recourse to the monographs by Thom and Raper where these fungi are copiously illustrated.

Some errors have been noted, including *Helminthosporium* for *Heterosporium* (p. 341), also some species have been given incorrect references. Coming from the Antipodes, I am interested to note that, as in the first edition, several species of *Mortierella* isolated from Victoria, Australia, are accredited to New Zealand. These countries seem often to be confused!

Since many diverse organisms isolated by a wide range of techniques have been obtained from soil, one of the difficulties with a book of this type is to know what fungi to include and what to exclude. Dr Gilman originally set out to 'place a tool in the hands of investigators that will enable them to identify the soil fungi which they may encounter in the course of their work'. He also states that he does not include the Zoopagaceae since these are forms that will not usually appear among the fungi that are found in plate cultures made from soil samples, but require a special technique for their study. Similar reasons would also apply to other groups, such as chytrids, nematode-catching fungi, Basidiomycetes, and plant pathogens, not normally encountered by plating techniques. However, one large group, the 'water moulds', are included in this book, though most were undoubtedly isolated by special techniques; and also all these groups are occasionally found by plating techniques. Perhaps if Dr Gilman should produce a further edition, and I hope he is able to do so, he could give us further information on the basis of inclusion of species in his book. A further point is that anyone seeking information on the geographic distribution of soil fungi should note that the references given in this book are far from complete.

Nevertheless, this book is a useful one and should find a place in the bookshelf of anyone interested in the identification of moulds and other lower fungi, whether isolated from soil or not.

J. H. WARCUP

The Chemistry and Biology of Yeasts. Ed. by A. H. Cook. (New York: Academic Press Inc.; London: Academic Books Ltd., 1958.) Pp. xii+763, 3 plates, 58 figures. Price: $22 or £7. 17*s*.

The knowledge that has accumulated from research on yeasts is now so vast and so technical that familiarity with any particular aspect is possible only for the specialist. This volume is, quite rightly, the work of a symposium of specialists, attempting to supply, in the words of its editor, 'a single, adequately integrated, up-to-date source of information'. That the field is widely covered is shown by the titles of the chapters: I. Classification by J. Lodder, W. C. Sloof and N. J. W. Kreger-van Rij; II. Ecology by A. Lund; III. Life history and Cytology; and IV. Genetics by Ö .Winge and C. Roberts; V. Aspects of the chemical composition by A. A. Eddy; VI. Growth by E. O. Morris; VII. Fermentation and respiration by F. F. Nord and S. Weiss; VIII. Synthesis and degradation of cellular carbohydrates by W. E. Trevelyan; IX. Nitrogen metabolism by G. Harris; X. Technology by M. Pyke; XI. Pathogenic Yeasts by G. C. Ainsworth; XII. Yeasts in food spoilage by M. Ingram; and XIII. Flocculation by H. E. Jansen. Each is followed by a comprehensive bibliography (2–12 pp.).

Here, then, is a very useful source book for those seeking an introduction to the subject, especially University students and those entering the industry, a handy reference for specialists not particularly familiar with aspects outside their own line of research, and an up-to-date survey in condensed form for those who wish to keep abreast of research but cannot read the multitude of original papers.

A review that would do justice to all the articles would need a symposium of reviewers. Here, it is necessary only to add a few words on the mycological aspects. Classification follows essentially the pattern of *The Yeasts* by Lodder and Kreger-Van Rij, 1952, but the authors have also given an impartial survey of recent taxonomic studies expressing views divergent from their own, as well as bringing their classification up to date. The article on ecology is full and interesting, though necessarily very condensed, but some aspects are covered in more detail in chapters XI and XII. Other chapters of interest to the mycologist are III and IV where much highly technical information is integrated into a readable account.

One serious drawback is that in different parts of the book the same yeast is referred to under two or even three binomials without any cross reference to indicate that they are the same. Where obligate synonyms have been used by the authors of the various articles the situation called for clarification in the index. Thus, there should have been only one series of entries for *Hansenula anomala*, *Saccharomyces anomalus*, *Willia anomala* and *Candida pelliculosa*, with appropriate cross references, to quote only one example of many. The situation becomes almost absurd when *H. saturna* and *H. saturnus* have separate entries.

The book is well produced on good paper with commendably few printer's errors, fewer indeed than authors' errors, but not many individuals in this country will, I imagine, pay the high price of such workmanship; it is to be hoped that it will find a place in libraries.

GRACE M. WATERHOUSE

Mycoses of Man and Animals. By R. VANBREUSEGHEM. English translation by J. WILKINSON. (London: Pitman and Sons Ltd., 1958.) Pp. 235, 48 figures. Price: 50*s*.

The *Précis de Mycologie*, which was first published by Langeron in 1945, was an attempt to cover the whole field of mycology, but only 77 pages were allotted to medical mycology. This subject was, at that period, beginning to expand rapidly, so that by the time the *Précis* entered its second edition the number of pages devoted to it was of necessity increased threefold. The re-editing of this book devolved mainly on Prof. Vanbreuseghem, a former pupil and assistant of the late Prof. Langeron who died shortly after he himself had embarked on the task. Prof. Vanbreuseghem's wide experience of both European and tropical mycology was reflected mainly in his treatment of the section on medical mycology. Besides including extracts from his own considerable contribution to this branch of mycology this section contained most of the more important results obtained by workers in many parts of the world. In this second edition, the mycoses were dealt with in alphabetical order, commencing with those caused by actinomycetes and ending with Sporotrichosis. The seventeen chapters which comprised this section have now been published as a separate book in English. The translator, Dr Wilkinson, has provided English-speaking readers with an attractively written text while avoiding the errors of misinterpretation. This is an essential requirement for a translation and Dr Wilkinson is to be congratulated on having fulfilled it to perfection. A few terms not in common usage amongst English-speaking medical mycologists may be picked out here and there in the text; such are: corneal, polynevritis, microsporia and tendrils. In the French text these are respectively; cornée, polynévrite, microsporie and vrilles. These minor defects will doubtless be corrected in a translation of a third edition which would seem to be even now overdue.

C. J. LA TOUCHE

Uredinées du Maroc I. By A. L. Guyot and G. Malençon. Travaux de L'Institute scientifique chérifien, Série botanique, No. 11. (Rabat: Société des sciences naturelles et physiques du Maroc, 17 May 1957.) Pp. v + 184, 4 plates, 29 text-figures.

This first contribution to a revision of the Uredinales of Morocco, based mainly on collections made by the authors between 1952 and 1956, includes the results of studies on a number of important species. It is planned to extend the work to cover the whole rust flora of the country. Six new species are described (with Latin diagnoses) and a number of new host records are given. Particular attention is paid to certain pleomorphic species, notably *Puccinia graminis*, and detailed descriptions, with spore dimensions, are given of a number of collections of such species from different hosts. Such data form valuable material for any future revision of the species concerned.

The results of biological studies on *P. graminis*, with special reference to its physiologic specialization, are to be given in a separate memoir. It is to be hoped that such studies will be extended to other species. Only by such a synthesis of morphological and biological studies will it be possible to elucidate the status of many of the varieties and forms of rust fungi described from the Mediterranean region.

J. G. MANNERS

The Genus Cortinarius. II. Inoloma *and* Dermocybe. By P. D. Orton. Pp. 68, 17 figures, 1958. (Issued by *The Naturalist* and obtainable from the editor, *The Naturalist*, The University, Leeds 2.) Price: 7*s*. 6*d*.

This is a continuation of Mr Orton's Study of the Genus *Cortinarius*, the first part, covering *Phlegmacium* and *Myxacium*, having been published in 1955. To some extent he has based his classification on M. Moser's recent work in *Kleine Kryptogamenflora*, IIb, Stuttgart, 1955, but with certain reservations and modifications, which, if only for purposes of identification, are undoubtedly to be recommended. He deals here with the subgenera *Dermocybe* and *Cortinarius* and his new subgenus *Sericeocybe*. Subgenus *Inoloma* is sunk because its type, *Cortinarius violaceus*, is the same as for *Cortinarius*.

Orton's main criteria for distinguishing these dry Cortinarii are cap colour, nature of cap surface and stem shape. In all fifty-eight species are keyed and are described in tabular form. Seven of these are new to science and sixteen are recorded for the first time in this country.

This concise and useful paper brings a great measure of order to a genus difficult on account of being homogeneous yet plastic, possessing as it does a large number of distinctive yet definitely 'cortinarioid' species.

FRANCES L. BALFOUR-BROWNE

The Mushroom Hunter's Field Guide. By Alexander H. Smith. (Michigan: University of Michigan Press, 1958.) Pp. 197, 156 photographs. Price: £2.

This is a beautifully produced book of a convenient shape (10 × 5 in.) for using in the field. It has been written for mycophagists (with little or no mycological training) collecting in the Great Lakes region, western and north-eastern United States. One hundred and twenty-four of the common species in this area are dealt with. Formal descriptions are not given and the author groups his comments on each species under three headings: 'When and where to find it'; 'Important Field Characteristics' and

'Edible' (or 'Poisonous'). A photograph illustrates every species, and while these are first class the inclusion of some coloured plates particularly for the genera *Lactarius* and *Russula* would have facilitated the identification of these fungi.

Over one-third of the species described in this book are not found in this country, conversely, many of our common species do not occur in the regions of America for which this book is written. Thus there is no mention of *Amanita phalloides* which could be unfortunate for British mycophagists. While this seems an excellent book for use in America, collectors in Britain will find it of limited value.

S. M. FRANCIS

Fungi of the Færöes. Part II. With an Appendix to Part I. By F. H. Möller. (Copenhagen: Ejnar Munksgaard, 1958.) Pp. 268, 161 figures, 1 col. plate. Price: Danish Cr. 60.

Part I of the *Fungi of the Færöes*, published in 1945, dealt with the basidiomycetes. Part II deals with the remaining groups and an Appendix gives supplementary records for rusts, smuts and larger basidiomycetes. In the preparation of this concluding volume the author has had the help of Dr A. Monk with the pyrenomycetes and of Prof. J. A. Nannfeldt with discomycetes, while Prof. N. F. Buchwald has been responsible for Fungi Imperfecti and other groups of micro-fungi.

The main part is a systematically arranged check-list. New taxa are fully described and for many others there are partial descriptions and notes on biology and geographical distribution. There are no keys but various tabulations, host and habitat indexes and in conclusion a general index to the two volumes make it easy to consult a work which should provide a firm foundation for further studies on the mycology of the Færöes.

G.C.A.

Advances in Genetics. Vol. IX. Edited by M. Demerec. (New York: Academic Press Inc.; London: Academic Books Ltd., 1958.) Pp. viii + 294. Price: $8.80.

This latest volume in a useful series contains three articles of considerable interest to mycologists. In the first, by G. Pontecorvo and Etta Käfer on 'Genetic analysis based on mitotic recombination' (pp. 71–104), which is a sequel to the one by Pontecorvo already noticed in these *Transactions* (**38**, p. 96, 1955), the authors describe the principles of mitotic analysis based on the parasexual cycle. In the second (pp. 105–146) Etta Käfer provides a worked example of the method culminating in a set of chromosome maps for all eight chromosomes of *Aspergillus nidulans*, which is surely as historic an event as the publication of the first single chromosome map of a fungus (*Neurospora sitophila*) by Lindegren in 1936. The third article (pp. 41–69), by H. P. Papazian, is on 'The genetics of basidiomycetes' with emphasis on *Coprinus* and *Schizophyllum*.

G.C.A.

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p. 246, Text-fig. 4. *For* 'cellulose' *read* 'cellulase'.

p. 248, Explanation of Plate 12, Fig. 2. *For* '0·1' *read* '0·01'.

INSTRUCTIONS TO AUTHORS

Contributors are asked to be clear and concise, and to use a recent issue of the *Transactions* as a guide to the arrangement of headings and sub-headings, explanations of plates and figures, references to the literature, and other details. Each paper should start with a short summary. Contributors are urged not to adopt long serial titles and sub-titles.

The standards adopted in the *Transactions* are those outlined in the Royal Society's pamphlet, *General Notes on the Preparation of Scientific Papers* (1950, The Royal Society, Burlington House, London, W. 1, price 2*s*. 6*d*.). An exception is that in the *Transactions* citations of references to literature, although based on the Harvard system, include the full titles of the papers and complete page numbers. Titles of journals are abbreviated in accordance with *A World List of Scientific Periodicals*, 1952.

The scientific names of genera and species are printed in italics and should be underlined in the typescript. The title of the paper, headings, and subheadings may be in capitals, but should not be underlined.

The attention of authors proposing new taxonomic groups is drawn to the need to give adequate descriptions and figures, and to designate the types as required by the *International Code of Botanical Nomenclature*. The description of a new species should be accompanied by a figure, and whenever possible the type specimen should be deposited in one of the national herbaria.

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